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MYANMAR ARMY HEALTH JOURNAL

Aims of the Journal:

Myanmar Army Health Journal is a professional medical and health science research journal to address the following aims and objectives:

1. To equip the members of medical corps by disseminating impartial biomedical information discovered nationally and internationally up to date in order to make soldiers healthy and fit to fight.
2. To inspire the military healthcare professionals to conduct researches relevant to the welfare and benefits of the soldiers and family members of Tatmadaw and the people of the country.
3. To support creating research culture and environment among and inclusive of experienced and inexperienced healthcare personnel.
4. To serve as a suitable ground and to provide opportunities to nurture research article writing skills and to archive the research works of military medical scientists.

NOTICE TO CONTRIBUTORS

Myanmar Army Health Journal welcomes contributions from home and abroad and publishes original articles, short papers, case reports and correspondence in the field of biomedical and health sciences. Papers are accepted for consideration on the understanding that their contents have not been published in whole or in part elsewhere, that they are subject to editorial revision and that the Editorial Board is responsible for the order of publication.

ORIGINAL ARTICLES

These should be headed with the title, the name(s) of the author(s), and the address (es) where the work was done. They should be accompanied by an abstract of not more than 300words which will precede the main text of the paper and should convey its scope. The articles should be arranged as follows:

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- (3) Results:
- (4) Discussion and Conclusions:
- (5) Acknowledgements and
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These should not exceed 1000 words. 2 illustrations or figures and 5 references and should include (1) Case Report: (2) Comment and (3) References.

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Letters to the editor, questions or comments on papers published in this journal, and, if appropriate, replies to comments are welcome. Letters should generally not exceed 350 words, tables and figure are not accepted. References (not more than 2) may be given only if essential.

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Illustrations (Tables, Graphs, Drawings, Figures, Photographs – not more than 5 each on a separate page)

Illustrations - Details of results presented in this way should not be repeated in the text. Each table should be numbered and complete with title and footnotes. Line drawings, maps and graphs should be in Indian ink on white paper, and of suitable size for reduction in black and white, glossy prints and approximate final size. Tables and legends to figures should be typewritten.

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Journal article – more than six authors	Hanna et al ²⁰ report in this article that ...	20. Hanna JN, McBride WJ, Brookes DL, Shield J, Taylor CT, Smith IL, Craig SB, Smith GA. Hendra virus infection in a veterinarian. Med J Aust. 2006 Nov 20;185(10):562-64
Standard book- One author	The book, “Secrets from the black bag”, by Butler ¹ shows that ...OR Butler ¹ purported ‘...’	1. Butler SW. Secrets from the Black Bag. London: The Royal College of General Practitioners;2005.
Standard book- more than six authors	Professionals collaboration ³ in	3. Hofmeyr GJ, Neilson JP, Alfirevic Z, Crowther CA, Gulmezoglu AM, Hodnett ED et al. A Cochrane pocketbook: Pregnancy and childbirth. Chichester, West Sussex, England: John Wiley & Sons Ltd; 2008.

The author is responsible for the accuracy of his references.

Editorial

Obesity

Obesity may be defined as an abnormal growth of the adipose tissue due to an enlargement of fat cell size (hypertrophic obesities) or increase in fat cell number (hypertrophic obesities) or a combination of both. In developed countries and some developing countries, obesity is a health problem. The connection between severe obesity and premature death from diabetes, hypertension and coronary heart disease is well established. The basic cause of obesity is over nutrition. A diet contains more energy than needed may lead to prolonged post-prandial hyperlipidemia to deposition of triglycerides in adipose tissue resulting in obesity.

Obesity is often expressed in terms of body mass index (BMI). Overweight is usually due to obesity but can arise from other causes such as abnormal muscle development or fluid retention.

Body mass index (BMI) is body weight in Kg divided by height in meter square. Normal BMI is 18.5 to < 25, BMI <18.5 is under weight and BMI 25 to 30 is overweight. BMI 30 – 40 is obese and BMI >40 is very obese.

Epidemiological Factors of Obesity

- Diet, high calorie and low nutrient dense foods
- Physical inactivity
- Age and sex
- Socioeconomic status
- Certain medical condition and medications
- Race and Ethnicity
- Smoking cessation
- Family history
- Genetic susceptibility
- Psychosocial factors
- Education
- Alcohol
- Endocrine factors

Global Data

- One billion overweight adult globally
- 300 million obese adults
- Two thirds of UK adults are overweight
- 22 % of men and 23 % of women among overweight are obese
- More than 200 million adults are over-weight and obese in EU

Health Problems associated with Obesity

- Respiratory system- obstructive sleep apnoea, restricted respiration disease
- Cardiovascular system – hypertension, cardiomegaly, coronary artery disease, congestive heart failure, peripheral vascular disease
- Endocrine/metabolic disorder – diabetes mellitus, hypercholesterolaemia, hypertriglyceridaemia
- Gastrointestinal system - fatty liver, gall stone

- Musculoskeletal system – osteoarthritis, back pain, joint pain
- Psychological disorder

Healthy Life Span

Healthy life span can reduce the prevalence of obesity and overweight. 7 golden rules in healthy life style are –

1. Physically active
2. Keeping a healthy weight
3. Eating a healthy diet
4. Maintaining the health cholesterol level
5. Keeping blood pressure down
6. Regulation of blood glucose level
7. Non-smoking

With the awareness of promoting physical activity and healthy diet and avoidance of risk factors such as smoking, drinking and betel chewing, we can reduce the obesity and premature death.

In our Tatmadaw, leaders encourage the physical activity, 5 days in the morning and 3 days in the evening for a week. In each time, we jog to 2 miles and so altogether 16 miles in a week to get stamina and fitness. Other 2 days' evening, we can play the sport that we like.

For diet, there is in sufficient amount both quantitatively and qualitatively given by supply and transport units. Therefore, according to our mission “Make Fit to Fight” and our motto “Sincerity, Sympathy and Safety”, we, soldiers will be physically and mentally fit and can avoid from obesity and overweight. So, all soldiers can perform their duties perfectly and affectively.

**Colonel Thein Zaw
Chief Editor
Deputy Commandant and Visiting Professor
Defence Services Medical Research Centre**

LEADING ARTICLES

MEASLES, VACCINATION AND IMMUNITY

Measles: Global burden

Measles is one of the highly contagious infectious diseases, caused by the measles virus, a single-stranded RNA virus of the Paramyxoviridae family. In 2011, the WHO estimated that 158,000 deaths were caused by measles. Although measles is primarily considered to be a childhood disease, it can affect people of all ages. In developed countries, death occurs in 1 to 2 cases out of every 1,000 (0.1% - 0.2%). In populations with high levels of malnutrition and a lack of adequate healthcare, mortality can be as high as 10%. The introduction of a live measles vaccine has been associated with a dramatic reduction in measles. However, despite the vaccination programs adopted by many developed countries and advancements towards the goal of measles elimination, outbreaks continue to occur every year around the world. Recent strides have been made toward global measles control using a 2-dose vaccination approach, but logistical and financial difficulties in sustaining the current mass campaign strategy in developing countries have resulted in a resurgence in measles and measles deaths. In addition, complacency and concerns about safety, along with philosophical and religious objections to vaccination, have resulted in measles being re-established as an endemic disease in many industrialized nations.

Measles in Myanmar

Despite of routine immunization with the first dose of measles vaccine in 1987 and supplementary immunization activities carried out every year, measles is still endemic in Myanmar. In 2010, second dose of measles vaccination was introduced officially. Although the coverage of both the first and second doses never reached the effective level of 95% in the townships where measles immunization was deployed, the mortality rate of measles was found to be reduced by 90% in 2009 compared to 2000. From 2011 to 2013, there have been reports of measles outbreaks all over the country including military institutions and the border areas among ethnic groups. In January 2015, a National Measles Rubella Vaccine campaign was conducted in two phases in Myanmar. The first phase was targeted on school children 5-7 years in approximately 45,000 schools, government, private and monastic schools. The second phase covering 65,000 villages/urban wards was conducted in February 2015 vaccinating children from 9 months to 5 years of age plus children missed out in school phase, children who don't attend school. Recently, measles outbreak occurred among Myanmar Military Cadets in Pyin Oo Lwin, Myanmar during February, 2017.

Clinical features

Most cases have occurred in unvaccinated individuals presenting with the classic clinical picture of descending macular papular rash, fever, and either cough, coryza, or conjunctivitis; consequently, they can be readily diagnosed. Two or three days after the start of symptoms, small white spots may form inside the mouth, known as Koplik's spots. A red, flat rash which usually starts on the face and then spreads to the rest of the body typically begins three to five days after the start of symptoms. Symptoms usually develop 10–12 days after exposure to an infected person and last 7–10 days. Complications occur in about 30% and may include diarrhea, blindness, inflammation of the brain, and pneumonia among others.

Laboratory diagnosis

Suspected cases can be easily laboratory confirmed following timely collection of specimens, usually a serum specimen assayed for the presence of measles-specific IgM and concordant epidemiological information. Laboratory confirmation of measles virus infection is a critical component of the surveillance required to support measles control and elimination programs. Though detection of measles virus-specific IgM by enzyme immunoassay (EIA) is the most widely used method to confirm measles virus infection, suspected measles cases in highly vaccinated populations may require additional testing. Inconclusive results obtained by IgM testing can be confirmed by detection of measles virus RNA by reverse transcription (RT)-PCR.

Measles serology

The presence of IgG antibodies to measles virus indicates the occurrence of the infection but does not distinguish between recent and past infection. Virus-specific IgM antibodies are first detected ten days and peak at about four weeks post infection. They may persist for more than seven months after acute infections. Based on the evidence that antibody avidity gradually increases after exposure to an immunogen, avidity of IgG antibodies can be used as a marker for distinguishing recent primary from long-term infections. Avidity describes the binding strength of a specific antibody to its antigen. Low-avidity IgG antibodies indicate a primary infection, whereas the presence of IgG antibodies with high avidity points to persistency or reactivation of infection. Sensitive and specific commercial kits are widely available for the detection and the quantification of measles antibodies, and enzyme immunoassay (EIA) is the most widely used test format.

Immunity to Measles

Lifelong immunity is generally reported after a wild-type measles infection, while in vaccinated people a waning immunity has been reported in correlation with lower levels or more rapid decrease of measles specific antibodies. Asymptomatic reinfections or disease were observed in subjects where the immune system was challenged by 2-vaccine doses, probably because of an inadequate response. The proportion of vaccinated 20 to 24-year-old patients in the last French outbreak was 4.8%. Furthermore, occasional spread from unvaccinated patients with measles to 2-dose vaccine recipients has recently been observed. The risk of measles complications makes it important to rapidly detect the immunological status of the contact patients suffering from measles in order to identify those not immunized and exposed, and to vaccinate when necessary.

Vaccine failure and IgG antibody avidity

A suspected measles case in a previously vaccinated individual can be classified as a primary vaccine failure (PVF) by measurement of low-avidity measles IgG antibody. Individuals with confirmed measles and a prior immunologic response to measles virus (reinfection) from either vaccination or natural disease that occurred at least 4 months before symptom onset can be identified by the presence of high-avidity measles IgG antibody. A measles virus reinfection that occurs in an individual who had measurable specific antibodies after documented vaccination constitutes a secondary vaccine failure (SVF). However, the vaccination history of some persons with confirmed reinfections can be unknown, and among those with one documented doses of vaccine, evidence of a protective titer of antibody to measles following vaccination is rarely available. Therefore, the term reinfection case (RIC) can be universally applied to a confirmed measles case in a person with high-avidity measles

IgG antibody. Serum samples collected at or near the onset of rash from RICs often have undetectable measles-specific IgM while high levels of measles-specific IgG are present. Therefore, the best method for case confirmation of a RIC is RT-PCR testing. However, measurement of high concentrations of measles neutralizing antibodies by the plaque reduction neutralization (PRN) assay may provide an alternative method to confirm RICs when standard laboratory tests are inconclusive.

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Current Laboratory Diagnostic Methods in Tuberculosis

Infectious diseases

Infectious diseases are the second leading cause of death and the leading cause of disability-adjusted life years worldwide¹. They are now spreading geographically much faster than at any time in history due to highly mobile, interdependent and interconnected nature of the world. Infectious diseases are not only spreading faster, they appear to be emerging more quickly than ever before. Since the 1970s, newly emerging diseases have been identified at the unprecedented rate of one or more per year. There are now nearly 40 diseases that were unknown a generation ago. The spread and emerging nature of infectious diseases are superimposed by the spread of antimicrobial resistance, especially with extensively drug-resistant tuberculosis (XDR-TB)².

The transition year

Now is the time when human are succeeding against tuberculosis. 2015-2016 is the transition year and watershed moment in the battle against tuberculosis, marking the deadline of the Millennium Development Goals (MDG) and the new era of Sustainable Development Goals (SDG). In other words, this is the change from the Stop TB Strategy to the End TB Strategy. Remarkable improvements were revealed by 18% fall of TB incidence since 2000 and 47% fall of TB mortality since 1990³.

TB problem

Despite these advances and despite the fact that nearly all cases can be cured, TB remains one of the world's biggest threats. Estimated about 9.6 million people worldwide had fallen ill with TB in 2014 with 58% occurring in the South-East Asia and Western Pacific regions. It still killed 1.5 million people in that year. TB now ranks alongside HIV as a leading cause of deaths globally³.

Another problem yet to be addressed was estimated 37% undiagnosed or unreported new cases. The quality of care for those people was unknown and there became a huge threat of the spread of disease and emergence of drug resistant strains³.

Drug-resistant TB is posing a major threat to control of TB worldwide. By the end of 2014, 153 countries were found to have anti-TB drug resistance. Globally, an estimated 3.3% of new cases and 20% of previously treated cases have MDR-TB. There were about 480000 multidrug-resistant TB (MDR-TB) cases in 2014 with 190000 deaths, but like the others, only a quarter were detected and reported and a major diagnostic gap persisted. It was worst in the Western Pacific Region where detected cases represented only 19% of estimated cases. People with MDR-TB or RR-TB are eligible for second-line treatment with MDR-TB regimens whereas undetected drug resistant cases will miss that treatment. The ratio of enrolled to notified MDR/RR-TB cases was 90% globally whereas it was <60% in 3 high MDR-TB burden countries including Myanmar (44%)³.

Extensively drug-resistant TB (XDR-TB) had also been reported by 105 countries by 2015. An estimated 9.7% of people with MDR-TB have XDR-TB (3).

Myanmar: one of the high burden countries

During the period 1998 to 2015, countries with high TB prevalence were categorized as “high burden country” (HBC) and listed as TB, TB/HIV and MDR-TB HBC. Myanmar was among all three lists but due to its active participation, all three targets of halving the TB incidence, prevalence and mortality rates in Myanmar, compared with 1990, were achieved at the end of MDG. But not to reduce efforts, Myanmar is still among 3 new HBC lists which

were redefined for the period 2016-2020. More importantly, it is also among 14 central diamond countries that are in all three lists³.

Role of laboratories

The End TB Strategy calls for the early diagnosis of TB and universal drug susceptibility testing (DST), highlighting the critical role of laboratories in the post-2015 era for rapidly and accurately detecting TB and drug resistance, which is essential to get timely and appropriate treatment³.

Microscopy

While inexpensive and requiring minimal biosafety standards, microscopy is not a sensitive test and it provides no information on the resistance profile of the bacilli. Furthermore, microscopy is not able to distinguish between *Mycobacterium tuberculosis* complex and non-tuberculosis mycobacteria³.

Smear microscopy remains the most widely used tool for TB diagnosis in low- and middle-income countries. But external quality assessment scheme was done only in nine out of 22 HBC countries with 13 countries lacking that scheme. In 2014, 50 out of 173 responding countries and territories still could not practice a formal quality management system towards achieving laboratory accreditation³.

Microscopy, which is the gold standard in malaria diagnosis⁴⁻⁷, has significant limitations in its performance in tuberculosis⁸. Specificity of ZN microscopy is high but sensitivity is variable (20-80%)^{9,10}. The sensitivity is grossly compromised when the bacterial load is less than 10,000 organisms/ml sputum sample⁸. It also has a poor track record in extra-pulmonary tuberculosis, paediatric tuberculosis and in patients co-infected with HIV and tuberculosis⁸⁻¹⁰. Due to the requirement of serial sputum examinations, some patients who do not come back for repeated sputum examinations become “diagnostic defaulters”. Some do not come back for results, and are lost to treatment and follow up. A personal observation showed that limited resources, large numbers of samples, all combined together often reduce the observation time per slide to less than 60 seconds, and this also contributes to reduction in the sensitivity of the test⁸.

Other laboratory tests

In clinical practice and surveillance program, laboratory diagnosis of tuberculosis by AFB microscopy is almost always supplemented by looking at rapid ESR and lymphocytosis. But the diagnostic value of ESR has been debatable as some studies found correlation between ESR and tuberculosis¹¹ whereas some indicated that ESR had little value as a diagnostic test for tuberculosis because a significant proportion of patients with TB had a normal ESR at the time of diagnosis¹².

Studies pointed out that although it was obvious of association between recent tuberculosis infection and peripheral blood lymphocytosis involving T lymphocytes, no changes were seen in the distribution of peripheral blood lymphocyte populations in previously diagnosed patients¹³. Others showed both lymphocytosis¹⁴ and lymphocytopenia. It was said that active tuberculosis causes a decrease in total T-cells secondary to a decrease in T4 cells¹⁵. Total B-cells are also decreased. Successful antituberculous treatment restores these values to normal¹⁶. Lymphocytosis as this is a normal immune response.

Serological tests

Extensive reviews and meta-analyses have concluded that the presently available antibody detection based serological tests are no good for the diagnosis of tuberculosis while

helpful for other diseases. In 2008, the WHO started a kit evaluation programme for various infectious diseases including HIV, hepatitis and malaria and TB rapid test kits. Only a few TB test kit firms responded to WHO evaluation programme and submitted their kits¹⁷.

For rapid diagnosis and discrimination between active tuberculosis and other pulmonary diseases, detection of serum immunoglobulin IgG and IgM antibodies raised against mycobacterial 38-kDa, 16-kDa, and 6-kDa antigens by a commercial rapid immunochromatographic IgG/IgM test is commonly used. Sensitivity of IgG in patients with active TB was somewhat high enough around 70% and specificity very elevated at 100%. So, IgG-mediated response can be used as a clinically useful tool for presumptive diagnosis and discrimination of active pulmonary TB from other pulmonary diseases. Based on its simplicity and rapidity of application, it could be a screening tool for active pulmonary TB in poorly equipped laboratories. But, because of its poor sensitivity, it cannot be used at well-equipped laboratories and in the community which demands high sensitivity. Although the specificity of IgM is the same as IgG at 100%, its sensitivity is much lower sometimes reaching to less than 5%. It cannot be used as a diagnostic tool at any place¹⁸.

Sensitivity is very critical and any test which has lesser detection rate than sputum microscopy does not warrant serious attention. The WHO advisory, based on exhaustive literature search and kit evaluation, found that the diagnostic sensitivities of these tests with patients with active tuberculosis ranged from as low as 16 per cent and maximum up to 57 per cent. Even the best kit has poorer impact on TB diagnosis than the most cost-effective and rapid smear microscopy. The sensitivity was no better for extra-pulmonary cases, an argument most often put forward in support of serology. About the specificity, as many as 28 per cent healthy contacts were found to be reactive to MYCO IgM, IgA, or IgG. A devastating cross-reactivity (up to 72%) was also found in kala-azar patients¹⁷.

The claims of every manufacturer that their product is better are extremely tall and misguiding. Indeed all these claims are based on in-house or small studies with no proper validation. A vast majority of studies were either sponsored by industry, involved commercial test manufacturers, or failed to provide information on industry sponsorship. Therefore, the WHO made a policy statement that commercial serological tests provide inconsistent and imprecise findings resulting in highly variable values for sensitivity and specificity adversely impacting patient safety. Overall data quality was graded as very low and it is strongly recommended that these tests should not be used for the diagnosis of both pulmonary and extra-pulmonary TB¹⁷.

The basic premise of serological tests was ease, rapidity and ever increasing demand in TB endemic countries. Hence, these tests have always been the first choice for small time laboratories wanting to mint the easy money from poor patients. Unfortunately, unethical medical practices provided major boost to these kits in recent years¹⁷.

Fluorescent LED microscopy

Now, it is found that fluorescent light-emitting diode (LED) microscopy is more sensitive than conventional Ziehl–Neelsen (ZN) light microscopy and has further qualitative, operational and cost advantages. But the switch to LED microscopes has been gradual globally as the technology was present in only 7% of microscopy centres in 2014³.

Bacteriological culture

Bacteriological culture is considered the reference standard for detecting TB, but suffers from the disadvantages that results take weeks to obtain and that testing requires a well-equipped laboratory, highly trained staff, and an efficient transport system to ensure the

viability of specimens³. Moreover, the organisms have to be present in the sputum to detect pulmonary tuberculosis.

Phenotypic DST on cultured specimens is the conventional method used to detect resistance to first- and second-line TB drugs. Recently, scientists invented rapid detecting liquid culture system using cutting edge incubation and detection technologies which can give result as fast as 3 days after inoculation. But building adequate culture capacity in many countries with a high burden of TB has been slow, given the cost and infrastructure requirements³. Deliberately multiplying tubercle bacilli also mandate the use of BSL-3 facility.

Molecular methods

In the diagnostics pipeline, tests based on molecular technologies are the most advanced. In recent years, a limited but growing number of rapid and more sensitive tests for TB and drug-resistant TB based on molecular methods, including Xpert® MTB/RIF and line probe assays (LPAs), have become available to replace or complement existing conventional tests³.

The use of the rapid molecular test Xpert MTB/RIF represents a major milestone for global TB diagnosis and care¹⁹, and it continues to expand in line with WHO recommendations for its use since December 2010. By the end of 2014, 69% of countries used it as the initial diagnostic test for people at risk of drug-resistant TB under the national policy. Likewise, 60% of countries used as the initial diagnostic test for people living with HIV³. The remaining countries still lack to perform initial diagnostic test for people at risk of drug-resistant TB or living with HIV³.

It uses world's renowned PCR system and microfluidic system. Now both infection and rifampicin resistance can be detected simultaneously within 120 minutes after docking of sputum inoculated cartridge at a module of the machine. Taking 15 minutes' sample processing time into account, total turnaround time is about 2.5 hours. Reliable diagnosis can be obtained through the use of this sensitive and specific test method. This method also guarantees the safety of the scientists and the environment as the bacteria has already been killed during treatment with NaOH/NOCl and close method is solely used in the whole process of DNA extraction, amplification and real-time fluorescent detection.

But GeneXpert can detect only rifampicin resistant gene (*rpoB*) at five commonly mutated sites. It is questionable if it can detect all mutation present all over the world. Epigenetics of the bacteria and body immune system also play the roles in combat of host against the agent.

Despite the advantages of molecular tests, conventional microscopy and culture remain necessary for monitoring patients' response to treatment. Furthermore culture-based DST methods are currently the only methods available for accurate testing of susceptibility to second-line drugs³.

Diagnostic gap

In 2014, only 58% of 52 million incident pulmonary TB patients notified globally could be confirmed by smear, culture or Xpert® MTB/RIF. The remaining 42% of patients who were not bacteriologically confirmed were diagnosed clinically, i.e. based on symptoms, chest X-ray abnormalities or suggestive histology. The common symptoms of TB combined with the poor specificity of X-ray screening may result in false diagnoses and people without TB being enrolled on TB treatment when it is not needed. Furthermore, a low rate of laboratory confirmation reflects under-diagnosis of true TB cases. Among new cases of

bacteriologically confirmed TB, 12% had access to DST where as 58% of previously treated cases had access to DST³.

Laboratory strengthening and new diagnostic methods are crucial to improve the proportion of notified TB cases with a definitive (bacteriologically confirmed) diagnosis of TB, and to close detection and treatment gaps for TB and drug-resistant TB³.

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ARTICLES

Study of G6PD Enzyme Activity in Myanmar Military Personnel

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Abstract: Glucose-6-Phosphate Dehydrogenase (G6PD) deficiency is the most prevalent enzyme deficiency, with an estimated 400 million people affected worldwide. Some of the drugs including Primaquine used in malaria can cause hemolysis in G6PD deficient patients. Since Soldiers are vulnerable to malaria, G6PD status of them should be determined prior to treating them with Primaquine. In this study, G6PD enzyme activity was determined by quantitative measurement of the enzyme activity in erythrocytes by using Randoxsemi-automated biochemical analyser based on ultraviolet spectrophotometric assay method at 340nm. The blood samples of 567 clinically healthy male soldiers from Tatkone military Units were collected with 3 ml EDTA containing tubes during 2015. The ages of participants were from 19 to 60 year old and mean age was 40 ± 10.68 year. The majority were Bamar 516 (91%). The mean enzymatic activity of all population was 6.35 ± 2.53 U/gHb. The population having enzymatic activity lower than 20% (<1.27 U/gHb) of mean enzyme activity were 7 (1.23%), those between 20% and 60% (1.27-3.81 U/gHb ~partial deficiency) were 64 (11.2%) and higher than 60% (>3.81 U/gHb) of mean enzyme activity were 496 (87.4%). The range of enzymatic activity in adult healthy male of Myanmar military population from Nay-Pyi-Taw was 3.82-8.88U/gHb. According to the upper cutoff point, the G6PD deficiency in our military population was 71(12.5%). By exploring and keeping data of G6PD deficiency status in Myanmar soldiers, the risk of haemolysis caused by administration of Primaquines and other 8-aminoquinolones can be reduced.

Keywords: G6PD, Primaquine, Myanmar soilders, spectrophotometry, malaria

1. Introduction

G6PD deficiency is the most common red cell enzymopathy estimated to affect 400 million people worldwide¹. A recent systematic review showed a global prevalence of 4.9% for G6PD deficiency². It is also a hereditary genetic defect, which is one of the most prevalent polymorphisms and enzymopathies in humans, particularly in males³⁻⁵.

The World Health Organization classifies G6PD genetic variants into five classes, the first three of which are deficiency states⁶.

- Class I: Severe deficiency (<10% activity) with chronic (nonspherocytic) hemolytic anemia
- Class II: Severe deficiency (<10% activity), with intermittent hemolysis
- Class III: Mild deficiency (10-60% activity), hemolysis with stressors only
- Class IV: Non-deficient variant, no clinical sequelae
- Class V: Increased enzyme activity, no clinical sequelae

In the study of Phompradit et al (2011), the prevalence of G6PD variants is 6.6% (21/317) in Myanmar population who resided in areas along the Thai-Myanmar border. Almost all (96.2%) of G6PD mutation samples collected from these Myanmar population carried G6PD Mahidol variant; only one sample (3.8%) carried G6PD Kaiping variant⁷. Than et al. (2005) reported the prevalence of G6PD Mahidol of 17.5% (160/916), while no carriers of the G6PD Viangchan mutation were detected in the population of malaria endemic southern Shan state of Myanmar⁸.

The geographical distribution of malaria is remarkably similar to the world distribution of deficient G6PD variants⁹. It is postulated that the high frequency of G6PD

deficiency has arisen because G6PD deficient variants confer some protection or resistance against malaria caused by *Plasmodium falciparum* and *Plasmodium vivax*¹⁰.

G6PD deficient individuals are asymptomatic until they are exposed to oxidative stress. Acute haemolytic anaemia resulted in these individuals from oxidative stress such as infection, drugs, chemicals and ingestion of some food stuffs¹¹.

Haemolysis in individuals with G6PD deficiency has been reported to follow therapy with a range of antimalarial drugs (8-aminoquinolines, including primaquine, tafenoquine and pamaquine), sulphones (dapsone), sulphonamides (such as sulphamamide, sulphamethoxazole and mafenide), analgesics (such as aspirin, phenazopyridine, and acetanilide), non-sulpha antibiotics (nalidixic acid, nitrofurantoin, isoniazid, and furazolidone), methylene blue and naphthalene¹. In addition, haemolysis can also be induced by foods (such as fava beans), henna, and infections (including Hepatitis viruses A or B, cytomegalovirus, pneumonia, and typhoid fever¹².

There is increasing public health concern for implementation of malaria elimination worldwide. Primaquine is the most important anti-malarial drug that can be used to eliminate *Plasmodium vivax* hypnozoites and *Plasmodium falciparum* gametocytes. Other anti-malarial drugs primaquine, dapsone, and the experimental drug tafenoquine are oxidative anti-malarials and can cause haemolytic anaemia¹³⁻¹⁵.

WHO recommends that if a patient is known to have severe G6PD deficiency (e.g. WHO G6PD deficiency class 2), primaquine should not be given since primaquine may cause massive hemolysis and even fatal¹¹. In mild-to-moderate GP6D deficiency primaquine might be given in dose of 0.75 mg base/kg body weight (bw) once a week for 6-8 weeks^{6,16-17}.

Screening for G6PD deficiency in vivax malaria patients is not usual test in Myanmar and there are no G6PD screening tests available in small military hospital laboratories. But some of these laboratories are situated in the malaria endemic area.

We, our soldiers are also lives in malaria endemic areas and no data about the prevalence of G6PD deficiency. Also the soldiers in Nay Pyi Taw and Yangon were experienced in living in that malaria endemic area during their frontline duties. There is justifiable to the diagnosis of these personnel who could be harmed by the administration of primaquine. Improvements to the diagnosis of G6PD deficiency are required for the broader and safer use of 8-aminoquinolines to kill hypnozoites.

Hypothesis

Genetically G6PD deficiency among our soldiers may be present. And it may be arisen in the soldiers because G6PD deficient variants confer some protection or resistance against malaria caused by *Plasmodium falciparum* and *Plasmodium vivax*

Justification

By detecting the G6PD deficiency in Myanmar soldiers, we can reduce the harmness caused by administration of primaquines and other 8-aminoquinolones in the malaria disinfection used.

2. Aim and objectives

Aim

To detect the G6PD enzyme activity of red cells in Myanmar soldiers.

Objectives

1. To study G6PD enzyme activity in the EDTA blood samples of soldiers from Nay-Pyi-Taw.

2. To find out the mean enzyme activity and the cases that lower than 60% of mean G6PD enzyme activity in Myanmar soldiers.
3. To correlate the G6PD enzyme activity within the different age group and ethnic groups.

3. Methods

Samples

The study was conducted during the 2015 in Military Units of Nay Pyi Taw. There were 567 soldiers enrolled for G6PD enzyme activity determination. The blood sample was then stored at 4°C in a refrigerator till required for use. All blood samples collected were analysed within 48 hours of collection.

Determination of G6PD activity

G6PD activity was determined by quantitative assay of the enzyme activity in erythrocytes based on Randox semi- automated biochemistry analyser by using the method of ultraviolet spectrophotometric assay. This is determined by measurement of the rate of absorbance change at 340 nm due to the reduction of NADP+. Calculation of G6PD enzyme activity in erythrocytes was as in the manufacture protocol. The haemoglobin of each blood sample was measured separately with hematological analyser and then calculated enzyme activity is expressed as U/gHb.

4. Results

The mean G6PD enzyme activity for the total population was 6.35 ± 2.35 U/gHb and normal range was 4.0 – 8.7 U/gHb. The population under 40 years age group (19-39yrs) was 47% with mean G6PD enzyme activity 6.44 ± 2.59 U/gHb and mean haemoglobin level of 13.8 ± 1.46 g/dl. The population above 40 years age group (40-60 yrs) was 53% with mean G6PD enzyme activity 6.26 ± 2.74 U/gHb and mean haemoglobin level of 13.66 ± 1.19 g/dl (table-1). There was no significant relation between age groups and different G6PD groups ($p=0.35$) (table 2). The mean G6PD enzyme activity and mean haemoglobin concentration in all ethnic groups were shown in table (3). There was also no significant relation between ethnic groups and G6PD groups. The age ranges from 19 years to 60 years old, mean age was 40 ± 10.68 years and all were male. The ethnic group in the study were Kachin10 (1.7%), Kayin 3 (0.5%), Chin 1 (0.2%), Mon 4 (0.7%), Bamar 516 (91%), Rakhaing 19 (3.3%), Shan 14 (2.4%). Mean G6PD enzyme activities and mean haemoglobin in each ethnic group were Kachin (n=10) 5.88U/gHb and 13.97g/dl, Kayin (n=3) 5.85U/gHb and 13.36g/dl, Chin (n=1) 4.78U/gHb and 11.5g/dl, Mon (n=4) 5.05U/gHb and 13.42g/dl , Bamar (n=516) 6.33U/gHb and 13.68g/dl, Rakhaing(n=19) 6.93U/gHb and 12.44g/dl, and Shan(n=14) 7.17U/gHb and 13.91g/dl. Mean G6PD enzyme activity was not correlate with mean Haemoglobin level within these groups ($p=0.2$). The population having enzymatic activity less than 20 % (<1.27 U/gHb) of mean enzyme activity were 7 (1.23%), those between (20 %) to (60 %) ($1.27\text{--}3.81$ U/gHb) were 64 (11.2%) and more than 60% (>3.81 U/gHb) of mean enzyme activity were 496 (87.4%). The upper and lower cutoff points for partial deficiency in our adult male soldiers were 3.81 U/gHb (60% of normal mean) and 1.27 U/gHb (20% of normal mean) (table 4).

Table (1) Age groups, mean G6PD enzyme activity and mean haemoglobin level

Age group	percentage	Mean G6PD enzyme activity (U/gHb)	Mean Haemoglobin level
19 to 39 yrs group	(262) 47%	6.44 ± 2.59	13.8 ± 1.46
40 to 60 yrs group	(305) 53%	6.26 ± 2.47	13.66 ± 1.19

Table (2) Age group and G6PD group

Age group	G6PD group		Total
	Equal and < 6.35U/gHb	'> 6.35 U/gHb	
19 to 39 yrs group	135	127	262
40 to 60 yrs group	169	136	305
	304	263	567

Table (3) Mean G6PD enzyme activity and mean Hb concentration in ethnic groups

No	Ethnic group	Number	Percentage	Mean G6PD enzyme activity U/gHb	Mean Haemoglobin g/dL
1	Kachin	10	1.7%	5.88	13.97
2	Kayin	3	0.6%	5.85	13.36
3	Chin	1	0.2%	4.78	11.5
4	Mon	4	0.8%	5.05	13.42
5	Bamar	516	91.0%	6.33	13.68
6	Rakhaing	19	3.3%	6.93	12.44
7	Shan	14	2.4%	7.17	13.91
	Total	567	100%	6.35	

Table (4) Distribution of G6PD enzyme activities

G6PD status	G6PD enzyme activity (U/g Hb)	Number of subject (%)
< 20% of mean enzyme activity	< 1.27 U/gHb	7 (1.23 %)
20% to 60 % of mean enzyme activity	1.27 – 3.81 U/gHb	64 (11.2 %)
> 60% of mean enzyme activity	>3.81 U/gHb	496 (87.4 %)

5. Discussion

Since 1956, it is well-known that the use of primaquine is not safe in people with erythrocyte glucose-6-phosphate dehydrogenase deficiency¹⁸. In this study we established the normal range, the mean and standard deviations for G6PD enzyme activity for healthy Myanmar military soldiers. In the study of Yang et al (2007), the mean G6PD enzyme activity is 6.47 ± 2.24 U/gHb for male and the study area is southwestern china¹⁹. This mean value is very closely related to our study results. In the study of RN Azma et al (2010), the sample size is 295 and the mean enzyme activity is 9.21 ± 2.6 U/gHb. They determined that the Cut-off point for partial deficiency as 5.52U/gHb (60% of normal mean) and the cut-off point for severe deficiency as 1.84 U/gHb (20% of norma mean)²⁰.

Table (5) The various studies of G6PD enzyme activities determination

Author	Year	Method	Place	Sample size	Results
Yang et al	2007	Nitroblue tetrazolium paper strip and G6PD/6GP-D	South-western China	490	Mean = 6.47 ± 2.24 U/gHb for male 5.83 ± 1.94 U/gHb for female <1.5 U/gHb is G6PD deficiency
RN Azma et al	2010	OSMMR-D Kit assay	Malaysia	295	Mean = 9.21 ± 2.6 U/gHb (Ref; 6.61 – 11.81 U/gHb) Cut-off point for partial deficiency 5.52U/gHb (60% of normal mean) and Cut-off point for severe deficiency 1.84U/gHb (20% of normal mean)
Saorin Kim et al	2011	Trinity Biotech (Modified spectrophotometric method)	Western Cambodia	903	Range 3.6 – 20.5 U/gHb Prevalence of deficiency in male = 15.6%
Duang-dao Nanta-komol et al	2013	Spectrophotometric biochemical assay	Thailand	295 Male 67 Female 228	Mean 11 ± 2.5 U/gHb in nondeficient female 10.9 ± 0.6 U/gHb in nondeficient male Cut-off point for partial deficiency 5.7 U/gHb Cut-off point for severe deficiency 0.95 U/gHb
Nicole laRue et al	2014	quantitative enzymatic colorimetric method	Westbury, NY	214	median G6PD activity for the entire population was 7.30 U/g Hb (range = 0.12–14.04 U/g Hb), and the adjusted male median was 7.18 U/g Hb (range = 0.84–12.26 U/g Hb)
Win Myat Oo et al (Present study)	2015	UV spectrophotometric assay	Nay Pyi Taw , Myanmar	567 all are male	Mean = 6.35 ± 2.53 U/gHb Cut-off point for partial deficiency=3.81U/gHb Cut-off point for severe deficiency=1.27U/gHb

Our cut-off points for partial deficiency can be used for the G6PD enzyme deficiency for the Myanmar military personnel and can be avoided for the incidence of haemolysis in the malaria patient taking the primaquine in these soldiers. In this study, we used the method of spectrophotometric assay for the determination of enzyme activity in erythrocytes based on Randox semi- automated biochemistry analyser. This method have many steps beginning with the elution stage for red cells lysis, followed by incubation of supernatant with reagents containing substrate and cofactor NADP and then the photometric measurement of the kinetic reaction at 340nm. The haemoglobin concentration of each sample is measured separately on an automated cell counter and G6PD enzyme activity is derived by manual calculation and expressed as U/gHb. Because of many preparation steps, accuracy can be reduced and time consuming. This can be the weak point of this study.

In the study of Saorin Kim et al (2011)²¹, they used modified spectrophotometric assay. The sample size was 903 and studied in western Cambodia. They got reference range 3.6 – 20.5 U/gHb and the prevalence of enzyme deficiency in male was 15.6%. Duangdao Nantakomo et al (2013)²² used spectrometric biochemical assay for the detection of G6PD enzyme activity in Thailand. In their study, 67 male and 228 female enrolled and mean G6PD enzyme activity in the non-deficient male is 10.9 ± 0.6 U/gHb. Cut-off point for partial deficiency is 5.7 U/gHb and cut-off point for severe deficiency is 0.95U/gHb. Their mean value is higher than our results but cut-off point for severe deficiency is not much different.

In the study of Nicole LaRue (2014)²³, the median G6PD activity for the entire population (n=214) was 7.30 U/gHb and the adjusted male median was 7.18 U/gHb. The study was done in Westbury, NewYork and based on colorimetric assay method. In Our study (2015), 567 male soldiers were involved and the findings of G6PD enzyme activities were concordant to the findings of Yang et al (2007) study (Table 5). Myanmar is a malaria endemic country and engaging in the malaria elimination now. Subsequently the introduction of primaquine for the radical cure of infection in the national malaria guideline is the mainstay of treatment. And also it can be a major safety challenge for policy makers. The field-adapted RDT for G6PD deficiency with accurate and user-friendly design should be easily available in our military community.

6. Conclusion

The mean G6PD enzyme activity for the total population was 6.35 ± 2.53 U/gHb. The upper and lower cut-off points for partial deficiency in our adult male soldiers were 3.81 U/gHb and 1.27 U/gHb. There were 1.3% of total population which have less than 20% of mean G6PD enzyme activity and 11.2% were less than 60% of mean enzyme activity. Since G6PD deficiency is the most common inherited blood disorder, readily available and inexpensive methodology for population screening of G6PD deficiency in our military community is vital for appropriate patient care. Our next step is to evaluate molecular genetic of G6PD deficiency in our military personnel. Our findings appear first to be documented report in military personnel with their G6PD enzyme activity.

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The Effectiveness of Innovative Method for Insecticide Impregnation of Mosquito-Net Used in Myanmar Armed Forces

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Abstract: The present study is to develop and establish new method for insecticide impregnation by characterizing and comparing two deltamethrin impregnation methods and quantitatively determining the deltamethrin retained in these mosquito nets. The conventional method being used in Myanmar Armed Forces at present is known as “dip-it yourself method” in which personal protection is important and causes laborious close contact with harmful insecticide. In this study, two mosquito-nets were impregnated with 10 ml of deltamethrin per 2 L of clean water (0.5 % v/v) by conventional “dip-it yourself method” taking 30 minutes for each mosquito-nets. Then, another two mosquito-nets were impregnated with the same concentration of deltamethrin by new innovative approach called “washing machine method” using 53L AW-A800MS Toshiba washing machine taking 10 minutes for two mosquito-nets simultaneously. Residual amounts of deltamethrin in mosquito-nets impregnated with both methods were determined using LC-20AD Shimadzu HPLC analyzer at 245 nm and compared. The measurement of insecticide for roofs and sides was performed because they were made of different textile materials. The results showed mean concentrations of 6.2 ± 0.2 mg/L compared to 15.2 ± 0.2 mg/L from roofs area, 9.3 ± 1.5 mg/L compared to 13.7 ± 3.8 mg/L from side areas and 8.9 ± 1.9 mg/L compared to 13.9 ± 3.5 mg/L for whole net, for conventional method and washing machine method, respectively, statistically significant ($P < 0.01$). This study demonstrated that higher quantity of deltamethrin residual amounts was detected in mosquito-nets using washing machine method compared to that of conventional method on their corresponding sides. Furthermore, this new method was convenient, time-saving and safer so that new innovative method should be considered.

Keywords: Mosquito nets, Deltamethrin, Impregnation, Conventional, Washing machine, HPLC.

1. Introduction

The first line of defence against mosquito-borne diseases, especially malaria is to prevent being bitten. This can be achieved by several integrated methods involving insecticides and repellents. One option is to reduce the biting density by killing adult and larval mosquitoes with insecticides and by using barrier insecticide impregnated mosquito-nets. These methods are suitable in static base locations, but not for protecting patrolling soldiers. In this situation, individual personal protective measures against mosquitoes are paramount; these include protective dress (long sleeves and trousers) and use of repellents and insecticide-impregnated mosquito-nets.¹

An insecticide-treated net (ITN) is a mosquito net that repels, disables and kills mosquitoes coming into contact with insecticide on the netting material. Insecticide treated nets usually target the adult vector.²

Mosquito-nets have been used for centuries as a barrier against biting mosquitoes. The use of mosquito-nets impregnated with insecticides was first trialled in Africa against malaria vectors in the mid-1970s. In the 1980s, a large number of trials were conducted globally using nets impregnated with synthetic pyrethroids — primarily permethrin and deltamethrin. In laboratory and field trials, insecticide-treated nets reduce the number of mosquitoes that successfully feed through the net and significantly increase the mortality in mosquitoes coming in contact with the net. Additionally, impregnation with insecticide significantly increases the protectiveness of damaged nets.³

The impregnated insecticide also acts to kill and repel any susceptible vector that rests on the net. ITNs act either as an adult mosquito control or reducing human-vector contact due to their combined effect. Large-scale implementation of ITN programme is part of an integrated approach to malaria control in many countries. In villages where impregnated nets are widely used, a reduction in vector density and longevity has often been observed.⁴

The results were encouraging and, in the mid-1990s, the use of insecticide-treated nets became the cornerstone of the World Health Organization's Roll Back Malaria initiative, aimed at halving the world's malaria by 2010.⁵

A conventional treatment of net is done by dipping in WHO-recommended pyrethroid insecticides such as deltamethrin, alpha-cypermethrin and permethrin.⁶ Deltamethrin is included in the chemical class of pyrethroids. Unlike other pyrethroids, deltamethrin consists of one pure compound. Deltamethrin impregnated mosquito net(s) (DIMN) is a newer weapon in the hands of the public health officials for control of malaria. It effectively combines the personal protective effect of a conventional mosquito net and the residual effects of Deltamethrin. Use of DIMN causes a decline in the mosquito density, biting density and longevity of the mosquitoes.

However, the use of deltamethrin insecticide can cause acute toxicity on oral, dermal after inhalation. Signs of toxicity in humans include paresthesia and skin sensations of tingling, itching, burning, and numbness after dermal exposure. Deltamethrin in cotton fields can exhibit dizziness, nausea, headache, fatigue, blurred vision, loss of appetite, sensations of burning and tingling in the face, vomiting, vertigo, disrupted sleep, twitching of muscles in arms and legs, convulsions, and also cause sensitivity to light, loss of bladder control, and loss of consciousness.⁷ Potential danger of chronic toxicity and tendency in endocrine disruption after exposure can also be occurred. And the worst effect may be having carcinogenicity and reproductive or teratogenic Effects.⁸

The residual persistence of insecticide may also depend on proper use of impregnated method. In the conventional impregnation method, the nets were carefully soaked in the standard insecticide concentrate in a "dip-it yourself" manner. In this method the personal protection were needed for safety of the person's health. And impregnation should be properly executed in order to ensure uniform distribution of the solution onto the net which is important to have longer residual persistence of the insecticide.

But if we are using another way of impregnation method with "washing machine", there could be least harmful to the person who carrying out such conventional method and could have advantages over conventional method in regard to have uniformity of distribution and prolong insecticide residual persistence.

Furthermore, the residual amount of insecticide can be lost or ineffective after six month storage time or after three consecutive washes. To ensure its persistent insecticidal effect, the net should be re-treated after three washes, or at least once a year.^{9,10}

2. Aims and Objectives

Aim

The aim of the study is to determine and compare the effectiveness of mosquito-net impregnation methods conventionally used in Myanmar with new innovative method of impregnation by using washing machine.

Objectives

- (1) To carry out deltamethrin impregnation on mosquito-nets by "dip-it yourself" method as conventional used of method.

- (2) To carry out deltamethrin impregnation on mosquito-nets by using “washing machine” as new innovative safe method.
- (3) To compare the residual amounts of deltamethrin on mosquito-nets impregnated by method conventionally used in Myanmar with impregnation by using washing machine as a new convenient method.
- (4) To determine the effectiveness of new mosquito-net impregnation method out of these two different impregnation methods.

3. Materials and Methods

Sampling method and sample size:

After impregnation with deltamethrin insecticide, for both methods using mosquito-net, samples will be taken as pieces randomly from multiple areas, from roofs and four sides (at different five areas; roofs, front sides, back sides, right sides and left sides). A sample was defined as one impregnated mosquito-net of (30 cm x 30) cm cut pieces randomly from each group. Sub-samples were taken by cutting 5 pieces again of (1 cm x 1 cm) in sizes; from above described different areas of the net, at recommended positions. Sampling as recommended procedure could leave sufficient material for other physical properties tests including dimensional stability, bursting strength, weight...etc.¹¹

Study design

This study was laboratory based cross-sectional comparative study from May 2015 to November 2015, done at Defence Services Medical Research Centre, Nay Pyi Taw.

Study Procedure

Military issued new fabric mosquito-nets in our country were used for both methods.

For impregnation with conventional method, The new mosquito-nets were impregnated with the doses of Deltamethrin equivalent to concentration 10 ml of deltamethrin per 2 L of clean water (0.5 % v/v) for each (one mosquito-net for one procedure) and two mosquito-nets included, as formerly instructed by the Military Health and Disease Control unit (HDC). The nets were carefully soaked in the standard deltamethrin concentrate in a “dip-it yourself” manner. The fingers were protected with plastic gloves and the nose was prevented with nasal mask. Impregnation was properly executed in order to ensure uniform distribution of the solution onto the net and was immersed for 30 minutes. Finally the impregnated mosquito-nets were stand dried in dark room for 24 hours.

For impregnation with new method using washing machine, 53 L AW-A800MS Toshiba washing machine was used. Two mosquito-nets were impregnated with equivalent to 200 ml of deltamethrin per 40 L of clean water (two mosquito-nets simultaneously), same concentration (0.5 % v/v) as “dip-it yourself” method. Then, mosquito-nets were spanned and immersed for 10 minutes. After that the nets were stand dried in same dark room for 24 hours.

Deltamethrin residual content analysis

The sample was extracted by acetone solvents and a mixture of acetonitrile and deionized water as mobile phase solution. The deltamethrin content was determined by normal phase high performance liquid chromatography and detection at 245 nm.

(a) Operating conditions were

- Mobile Phase: acetonitrile + deionized water = 85 + 15
- Flow rate 1.2ml/min, low power gradient
- Column temperature 40 °C (column oven)

- Inject Volume 20 μ l
- Run time 10 minutes

(b) Calibration

- Daily calibrated by HPLC system with full series of 6 standards, beginning with the lowest level standard.

(c) Preparation of sample

Was done by

- Weighed (to the nearest 0.1 mg) into a screw cap neutral glass bottle (10ml) sufficient sample to contain about 0.3 g of deltamethrin. Added 10 ml of Acetone (extract solvent). After that the cap was replaced closely.
- Put the bottles into the heater, setting temperature 100°C, running time 10 min. Then vigorously shake the bottle using the vortex mixer 5 min at room temperature, speed of shaking is at level of 2000-2500 beats per minute.
- Collect organic layer and again heat to dry. Then reconstitute with mobile solution 10 ml.
- Use syringe membrane filter with pore size of 0.45 μ m, filter for two times. 3ml extract solution was collected into clean screwed cap.
- Sample was injected within 24 hours since extraction, for longer waiting time, the caps were kept in a refrigerator.

(d) Determination

- Inject sample with HPLC and determining unit were concentration of mg/L.

(e) Linearity

- Over the range of Deltamethrin from 2 to 18 mg/L, the correlation coefficient R was 0.9993770 and superior linearity R^2 was 0.9987544 observed on first day, Test Method Summary.¹²

Data analysis

Data analysis will be done by SPSS biostatistics software version 19. Descriptive statistics will be used to determine the mean of residual persistence of deltamethrin on nets impregnated by two different impregnation methods and compare the residual amount of deltamethrin on mosquito-nets by independent samples *t* test.

4. Tables and Figures

4.1 Tables

Table (1) Amount of deltamethrin on nets impregnated by conventional method

Area	Samples					Mean (mg/L)
	1	2	3	4	5	
Roof	6.1	5.9	6.3	6.3	6.2	6.2
Front	7.8	7.4	7.4	8.6	7.6	7.8
Back	8.7	8.3	8.3	8.9	9.0	8.6
Right	12.0	11.3	11.9	11.3	11.1	11.5
Left	10.1	10.0	10.1	10.7	10.2	10.2

Table (1) showed detected amount of deltamethrin on samples at different areas by using conventional method. Five times sampling results were, (6.1, 5.9, 6.3, 6.3 and 6.2) mg/L in roof side, (7.8, 7.4, 7.4, 8.6 and 7.6) mg/L in front side, (8.7, 8.3, 8.3, 8.9 and 9.0) mg/L in back side, (12.0, 11.3, 11.9, 11.3 and 11.1) mg/L in right side and (10.1, 10.0, 10.1, 10.7 and 10.2) mg/L in left side, respectively.

Table (2) Amount of deltamethrin on nets impregnated by using washing machine method

Area	Samples					Mean (mg/L)
	1	2	3	4	5	
Roof	15.0	5.0	15.5	15.3	15.3	15.2
Front	17.6	6.6	17.9	18.0	17.5	17.5
Back	9.6	8.9	9.3	9.8	9.8	9.5
Right	17.4	6.9	17.1	17.8	17.1	17.3
Left	10.2	1.0	10.3	10.2	10.8	10.5

Table 2 showed detected amount of deltamethrin on samples at different areas by using washing machine method. Five times sampling results were, (15.0, 15.0, 15.5, 15.3 and 15.3) mg/L in roof side, (17.6, 16.6, 17.9, 18.0 and 17.5) mg/L in front side, (9.6, 8.9, 9.3, 9.8 and 9.8) mg/L in back side, (17.4, 16.9, 17.1, 17.8 and 17.1) mg/L in right side and (10.2, 11.0, 10.3, 10.2 and 10.8) mg/L in left side, respectively.

Table (3) Comparison of residual amounts of deltamethrin at roof and sides of Mosquito-net by two insecticide impregnation methods

Area	Conventional method Mean (mg/L)	Washing machine method Mean (mg/L)	t	p
Roofs	6.2±0.2	15.2±0.2	-73.975	< 0.01
Sides	9.3±1.5	13.7±3.8	-4.502	< 0.01
Whole nets	8.9±1.9	13.9±3.5	-6.463	< 0.01

After statistical analysis, the results showed mean concentrations of 6.2 ± 0.2 mg/L compared to 15.2 ± 0.2 mg/L from roofs area, 9.3 ± 1.5 mg/L compared to 13.7 ± 3.8 mg/L from side areas and 8.9 ± 1.9 mg/L compared to 13.9 ± 3.5 mg/L for whole net, for conventional method and washing machine method, respectively. These values were significantly different, ($P<0.01$) as shown in table 3.

4.2 Figures



Figure 1: Insecticide impregnation with conventional method

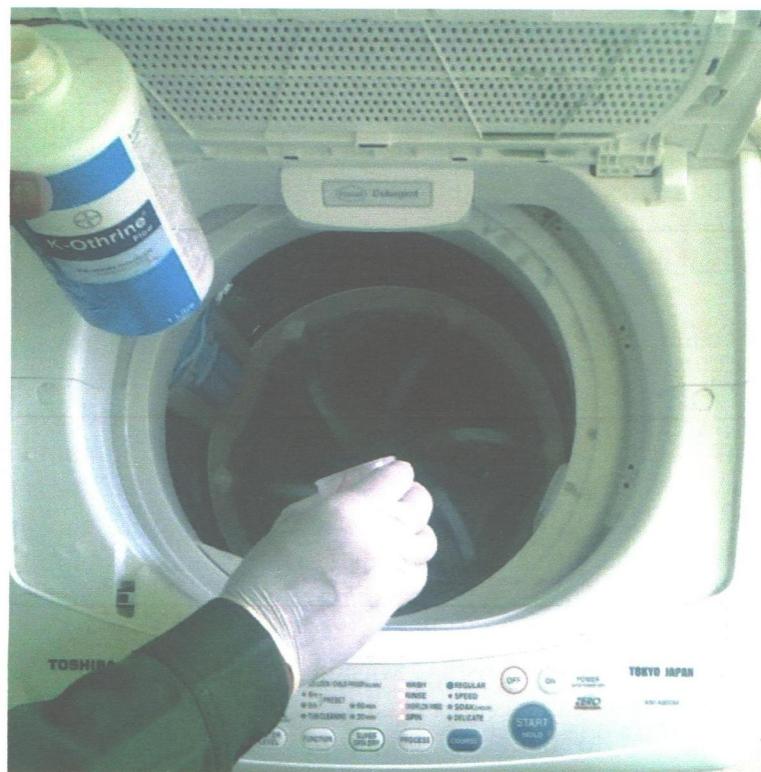


Figure 2: 53L AW-A800MS Toshiba washing machine used for new impregnation method

5. Equations and formula derivations

From the formula $N_1V_1 = N_2V_2$, ppm solution were dissolved and made preparation of calibration curves as follow—

Table(4) Preparation of calibration curves

Codes		Deltamethrin Standard	Extract Standar d	Stock (mg/ml)	Total Volum e
C1	18 ppm	30 μ l	970 μ l	0.018	1 ml
C2	10 ppm	17 μ l	983 μ l	0.010	1 ml
C3	8 ppm	13 μ l	987 μ l	0.008	1 ml
C4	6 ppm	10 μ l	990 μ l	0.006	1 ml
C5	4 ppm	7 μ l	993 μ l	0.004	1 ml
C6	2 ppm	4 μ l	996 μ l	0.002	1 ml

From the mixture of deltamethrin standard stock solution, different concentration levels of calibration solutions were made as shown in table 4 and put into a series of clean 10ml screw cap bottles after filtered through 0.45 μ m syringe filter before use.

6. Results and Discussions

Carrying out of deltamethrin impregnation on mosquito-nets by “dip-it yourself” method as conventional used of method was first introduced in present study. For that, the personal protection procedures were prepared first. The fingers were protected with plastic gloves, the nose with nasal mask and dressed uniform to enclosed limbs carefully. The personal protective measures were essential to the person health and impregnation properly executed by standard procedure, 10 ml of deltamethrin (2.5%) dissolved thoroughly into 2 L of clean water for one mosquito-net (0.5 % v/v) in order to ensure properly impregnation over nets. For doing properly, conventional method was skill needed. Otherwise, uniform distribution of the solution onto the net could not have and neither for enough retained amounts.

During procedure the insecticide mixture should not come into contact with the skin, particularly the lips, mouth, eyes and any open wounds and should be taken to avoid splashing into the eye and any open wounds. No clinical signs and symptoms detected in present study, where in other studies, paresthesia was the most commonly reported symptom from dermal exposure in occupational studies, skin sensations were characterized as tingling, itching, burning, and numbness of the skin after dermal exposure and the paresthesia was reported to be transient and reversible in a period of hours, sometimes lasting up to 48 hours.^{13,14} Paresthesia is considered to occur only at the site of dermal exposure and is not associated with systemic intoxication.¹⁴

Carrying out of new method using “washing machine” and with same concentration as conventional method was safe as it lacked laborious contact with the person and time saving, 10 min compared to that of 30 min in previous procedure. More amount of deltamethrin solution was used as more amount of clean water was needed to spin. As in Stephen PF and Robert DC study, they concluded that applying deltamethrin to mosquito-nets was a safe and effective way to increase protection against vectors of disease like malaria.¹⁵

The chromatographic HPLC technique was used for estimation of deltamethrin extracted by acetone solvents from the net, acetonitrile/water as the mobile phase and

detection at 245 nm in present study like other study done by Tyagi A, Sharma T, Singh M, Fatma K, Rawat VS, Aggarwal M and Khandal K.¹² Otherwise, detection at 235 nm in other study done by Gedeon YA, Patricia MG, Stephen CS, Aprielle BW, Mesele D, Tekola E *et al.* for HPLC technique with acetonitrile/water extraction of deltamethrin persistence estimation to PrmaNet.¹⁶

From of the mixture stock solution, different concentration levels of (0.002, 0.004, 0.006, 0.008, 0.010, 0.018 mg/mL) were prepared by appropriate dilution with extraction solvent to form the calibration curve solutions in present study and in contrast with (0.01, 0.1, 0.5, 1, 3, 5, 7 µg/mL) were prepared in other study.¹⁷

For the determination of residual amount retained in mosquito-nets, mean concentrations of 6.2 ± 0.2 mg/L compared to 15.2 ± 0.2 mg/L from roofs area, 9.3 ± 1.5 mg/L compared to 13.7 ± 3.8 mg/L from side areas and 8.9 ± 1.9 mg/L compared to 13.9 ± 3.5 mg/L for whole net, for conventional method and washing machine method, respectively. These values were statistically significant, ($P < 0.01$). The higher concentrations in new method may be due to more uniformly distribution of soaked chemical by using washing machine.

For contrast with other studies, the acetone extract of net samples contained mean concentration of 103 mg/kg of deltamethrin with HPLC.¹² The mean concentration of the insecticides were 5 g/kg (200 mg/m²) for InterceptorW nets, 1.83 g/kg (55 mg/m²) for PermaNetW2.0 nets and 1.8 g/kg (79 mg/m²) for NetprotectW nets by gas chromatography with electron capture detection (GC-µECD).¹⁶ Even the unit expressed was different (mg/L of 0.3 g weigh pieces of mosquito-net in present study), it was comparable for results taken from different areas of mosquito-nets using two different impregnated methods in present study.

7. Conclusion

This study demonstrated that higher quantity of deltamethrin residual amounts was detected in mosquito-nets using washing machine method compared to that of conventional method. Impregnation with new innovative method had more advantages in having more retained amount of insecticide chemical over mosquito-net lead to have reliable protective effect from mosquito bites. And the new method was convenient, time-saving and safer for the person carrying out such method.

According to findings in this study, we should consider replacing conventional “dip-it yourself method” with new innovative method called “washing machine method” as for the recommendation.

For suggestion to be made, the further studies should be done to find out the effect of washing on persistence of deltamethrin-treated mosquito-nets impregnated by two methods. And on the lost amount of Deltamethrin residuals on mosquito-nets impregnated by two different methods after wash; even more amount of deltamethrin impregnated solution was used for washing machine method, we should find of how longer that retained in mosquito-net by using “washing machine” method over “dip-it yourself” method as studies on cost-effectiveness. Then, studies should be on residual persistence after three-consecutive wash times even that is before six-month duration or after six-month duration of storage time to find out their life expectancy. Furthermore, the studies should be made on mosquito bioactivity and resistance to mosquito-nets.

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The Assessment of Available Biochemical Parameters in Adult Male and Female from Defense Services Medical Research Centre

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Abstract: According to the instruction of Directorate of Medical Services, all military personnel and family members should undergo medical checkup every four months. In the present study, authors investigated the available biochemical parameters such as random blood sugar, total protein, albumin, globulin, total bilirubin, AST, ALP, total cholesterol and triglycerides in 60 selected subjects, 33 males and 27 females (over 40 years of age) from Defense Services Medical Research Centre. These biochemical parameters were performed using Cobas (C-311) fully automated biochemical analyzer. The results showed that 24 (45%) subjects were within normal limits and the remaining 36 (55%) subjects were beyond normal limits. Among them, 17(28%) subjects had increased BMI (>25) and 3 subjects were chronic alcoholics. It was found that blood glucose level increased in 4 subjects (6.6%), triglycerides increased in 17 subjects (28 %) and total cholesterol increased in 26 subjects (43%). Two out of 3 chronic alcoholics had increased cholesterol level. Among 17 subjects with increased BMI, 5 subjects were increased cholesterol, 4 subjects were increased triglyceride and 5 subjects were increased both cholesterol and triglyceride. In this study, the increased BMI subjects had 2.4 times risk to increase cholesterol level than normal subjects. However, it did not reach the significant level. In conclusion, these available biochemical parameters highlighted to get early diagnosis and the intensity of impending disease among apparently healthy subjects. We can also give health education to promote healthy life style and suggest further management for increased BMI subjects.

Keywords: DSMRC, Biochemistry tests, BMI, Chronic alcoholism

1. Introduction

According to the instruction of Directorate of Medical Services, all military personnel and family members should undergo medical checkup every four months. The World Health Organization defines obesity as a condition with excessive fat accumulation in the body, to the extent that health and wellbeing are adversely affected.¹ Obesity is complex, multifactorial, environmental, social and cultural, genetic, physiological, metabolic, behavioural and psychological components. Obesity is affecting millions of people globally. It has been reported that type II diabetes, cardiovascular diseases, breast and colon cancers are related to obesity.²

Not only in western countries but also in Asia and African countries, obesity is still a problem. Obesity is increasing in China, more common among women³. In Japan, Obesity in men is common, adult obesity is increasing in men over 30 years and in women over 40 years⁴.

A typical Myanmar diet for middle class seems to be favorable in the prevention of cardiovascular disease which is low in animal fat and high in complex carbohydrates. Nowadays, eating fast food is getting popular in Myanmar and many people take these foods at least once a week.⁵

In the present study, authors investigated the available biochemical parameters such as random blood sugar, total protein, albumin, globulin, total bilirubin, AST, ALP, total cholesterol and triglycerides in 60 selected subjects, 35 males and 25 females (over 40 years of age) from Defense Services Medical Research Centre.

2. Objectives

1. To record the status of BMI of males and females (over 40 years of age) from Defense Services Medical Research Centre.
2. To determine the random blood sugar, total protein, albumin, globulin, total bilirubin, AST, ALP, total cholesterol and triglycerides levels in these subjects.
3. To determine the association between BMI and these biochemical parameters in these subjects.

3. Research Methodology

Study design

Descriptive study

Study Population

60 selected subjects, 35 males and 25 females (over 40 years of age).

Study area

Defense Services Medical Research Centre

Study Period

August 2015 to October 2015

Sampling method

Sampling method is simple random sampling method.

Methods

Random blood sugar, total protein, albumin, globulin, total bilirubin, AST, ALP, total cholesterol and triglycerides were performed using Cobas (C-311) fully automated biochemical analyzer

Data analysis

Data analysis was done by Epi Info Version 7 and SPSS 22.

Ethical consideration

Ethical clearance was obtained by Institutional Review Board of Defence Services Medical Research Centre.

4. Results

The results showed that 27(45%) subjects were within normal limits and the remaining 33(55%) subjects were beyond normal limits. Among them, 17(28%) subjects had increased BMI (>25) and 3 subjects were chronic alcoholics. It was found that blood glucose level increased in 4 subjects (6.6%), triglycerides increased in 17 subjects (28 %) and total cholesterol increased in 26 subjects (43%). Two out of 3 chronic alcoholics had increased cholesterol level. Among 17 subjects with increased BMI, 5 subjects were increased cholesterol, 4 subjects were increased triglyceride and 5 subjects were increased both cholesterol and triglyceride. There was significant association between increased BMI and cholesterol as well as triglyceride ($p<0.01$).

5. Conclusion

In conclusion, these available biochemical parameters highlighted to get early diagnosis and the intensity of impending disease among apparently healthy subjects. We can also give health education to promote healthy life style and suggest further management for increased BMI subjects.

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Rapid Quality Assessment of Different Brands of Anti-malarial Drugs by Thin Layer Chromatography

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Abstract: The proliferation of the fake, counterfeit and sub-standard, poor-quality drug is a major public health problem especially in developing countries. Thin layer chromatography (TLC) method could verify the drug's identity and quality rapidly and thus detect fake medicines by employing inexpensive analytical techniques. This study was aimed to assess the quality of different brands of widely used anti-malarial drugs bought from local registered pharmacy by TLC method. Two different brands of mefloquine 250 mg, two brands of artesunate 60 mg, two brands of dihydroartemisinin 40 mg and piperaquine phosphate 320 mg fixed dose combination tablets, two brands of artemether 20 mg and lumefantrine 120 mg fixed dose combination tablets were detected from September to October 2016 in Food and Drug Administration Department of Defence Services Medical Research Center. They were performed through the determination of quality control parameters of visual inspection, weight uniformity test, disintegration test and TLC profiles in comparison with their reference standards according to Global Pharma Health Fund (GPHF) minilab manual. The results revealed that 100% of all different brands of anti-malarial drugs complied with the tests of visual inspection, weight uniformity, disintegration, presence of active ingredient, absence of contaminant spots, as well as falling within the Rf range (retention factor range in chromatography) on TLC spotting in comparison with the reference standards. In conclusion, all different brands of anti-malarial drugs had quality of British Pharmacopoeia standard range. Therefore, standard quality control parameters should be monitored to check counterfeits and poor-quality of medicines in Myanmar not only for registered anti-malarial drugs but also for other kinds of drugs in the market for getting standard quality medicine.

Keyword: quality control parameters, counterfeit medicines, anti-malarial drugs, GPHF minilab

1. Introduction

The incidence of counterfeiting pharmaceutical products and proliferation of substandard quality medicines has been well identified internationally and constitutes serious health hazards. The World Health Organization has reported that counterfeit medicines potentially make up more than 50% of the global drug market, with a significant proportion of these fake products being encountered in developing countries. It is primarily flourishing in developing countries where institutional capacity in regulation, inspection and law enforcement is weak and adequate funds for regular drug quality monitoring are missing¹.

Counterfeiting of pharmaceutical products can take all kinds of form, but the end result is that the consequences range from treatment failure, increased toxicity, increased drug resistance and even outright death as a result of any of the above.

The prevalence of counterfeit medicines ranges from less than 1 percent of sales in developed countries, to over 10 percent in developing countries, depending on the geographic area. Africa, parts of Asia, and parts of Latin America have areas where more than 30% of the medicines on sale can be counterfeit. The trust of patients into health care and medicines is gradually fading away^{1, 3}.

Stumpf and Chaudhry also explored the effect of country on the counterfeit trade. In 2006, most (54%) of the counterfeit drugs detected were manufactured in India, with China manufacturing 21% and Hong Kong manufacturing 10%. Many countries in Asia with the geographical proximity to the counterfeit producers are deeply affected².

Owing to the widespread danger of counterfeit medicines, quality control in the distribution system of developing countries has acquired new dimensions today. However, pharmacopoeial analyses have become more and more expensive and only a few centers of excellence in some countries are currently available to perform them. Therefore, the development and use of simple tests should facilitate a balance between the need to increase the drug testing on the one hand and the need to contain costs on the other³.

The Global Pharma Health Fund (GPHF) mini-laboratory could verify the drug's identity and content and thus detect fake medicines by employing inexpensive analytical techniques. Once substandard quality medicine has been identified, some health care providers might decide not to pay the bill, some others might change the supplier in silent. Minilab can trigger off further investigations and instantly protect patients against treatments with ineffective counterfeit medicines³.

Malaria continues to be one of the major public health problems in Africa, Asia and Latin America. *Plasmodium falciparum* malaria is estimated to be the direct cause of 500 million cases and over 1 million deaths per year⁴. The recent emergence of ACT (artemisinin based combination therapy) -resistant *P. falciparum* malaria on the Southeast Asian region is of very great concern⁵. Poor-quality anti-malarials have been a severe under-recognized public health problem, reducing the effectiveness of these drugs and threatening current treatment policies.

Counterfeit anti-malarial drugs can be detected by technologies such as Raman spectroscopy, liquid chromatography mass spectrometry (LCMS), high performance liquid chromatography (HPLC), Fourier-transform infrared spectroscopic imaging, colorimetric assays and thin layer chromatography⁶. Among these, thin layer chromatography is simple and inexpensive. Several samples can be analyzed simultaneously. Thin layer chromatography has great flexibility in terms of stationary and mobile phases. TLC has no detection problems in the case of non-elution, thermal instability as HPLC and GC. The results detected by TLC are easy to interpret.

In addition, safety and efficacy of a pharmaceutical dosage form can be guaranteed when its quality is reliable. Generally, the efficacy of pharmaceutical dosage forms depends on their formulation properties, and manufacturing methods, hence it is likely that the quality of dosage form may vary⁷.

Currently, there are no reliable statistics on the level of incidence of fake drugs in Myanmar. The previous study indicated that counterfeit drugs existed in Myanmar: the overall failure rate in the case of the 214 samples was 16%⁸.

This study is carried out to assess the incidence of counterfeiting (if any) of most widely used anti-malarial drugs on sales in registered pharmacies within the Nay Pyi Taw capital, according to the World Health Organization's standards based on among others, methods in the Global Pharma Health Fund Minilab (2008).

2. Materials and Methods

Volumetric flasks, conical flasks, measuring cylinders, mortar, pestle, aluminum foil, pipettes, laboratory tubes, development chamber for TLC, filter paper, glass micro-capillaries, 10× 20 cm chromatography plates, methanol, acetone, glacial acetic acid, toluene, iodine chamber, sulphuric acid, ethyl acetate, ammonia, anti-malarial reference standard drugs, Ultraviolet fluorescence analysis cabinet (model CX-20), weight balance.

Two different brands of mefloquine 250 mg, two brands of artesunate 60 mg, two brands of dihydroartemisinin 40 mg and piperaquine phosphate 320 mg fixed dose combination tablets, two brands of artemether 20 mg and lumefantrine 120 mg fixed dose combination tablets were collected from registered pharmacies in Nay Pyi Taw capital. They

were coded into A1 and A2 for two brands of mefloquine, B1 and B2 for two brands of artesunate, C1 and C2 for two brands of dihydroartemisinin and piperaquine fixed dose combination, D1 and D2 for artemether and lumefantrine fixed dose combination tablets by simple random sampling method. Anti-malarial reference standards were obtained from DSMRC.

Visual Inspection

This involved an inspection of the following parameters; shape (circular, oval, flat sides, doubled layers, other), uniformity of shape, uniformity of color, no physical damage(cracks, breaks, abrasions, sticky), other observations (no foreign contaminant, dirty marks, proper seal) and the presence of batch number, manufacturing date, expiry date. The basic tests conducted were weighted based on WHO categorization of genuine, substandard and counterfeit medicines.

Weight Uniformity Test

20 tablets in each group of tablets were weighed individually (x) and also collectively and the weight recorded (Σx). The mean, of each group was then calculated and 5% of the mean was calculated. The weight variation was then calculated as Mean \pm 5% of the mean. If two tablets out of 20 are outside the range, the tablets were considered to have failed the weight variation test. The tablets were then crushed into powder and stored in airtight containers.

Simple Disintegration Test

The simple disintegration test was done in order to detect false claims on modified-release systems and defects on tablet and capsule hardness. In practice, place one tablet into a wide neck bottle containing a 100 mL of water of 37°C. For a precise measurement on temperature, an alcohol thermometer comes with the GPHF-Minilab. In order to imitate body movements when digestion medicine, stir or shake the liquid from time to time and continue to operate like this for 30 minutes. The test might stop earlier if the tablets under investigation were disintegrating much faster. Repeat the test with five more tablets respectively. The batch passed the test if all six tablets disintegrated. Repeat the entire test cycle should one tablet fail to disintegrate. Reject the batch should a further tablet fail again in the second and third run. All the tablets were expected to dissolve within 30 minutes and those that did not were considered to have failed the test.

Thin Layer Chromatography

The quality assurance of anti-malarial drugs was done by Thin Layer Chromatography. For analysis of mefloquine (A1 & A2), mefloquine tablets 250 mg was dissolved in 25 ml of methanol. 1 ml of the above supernatant liquid was then transferred into 3 ml of methanol. The expected concentration of mefloquine in this working sample solution was 2.5 mg per ml. This was also done using 250 mg of the pure mefloquine reference standard, after which spotting was done on normal chromatography plates coated with silica gel and developed in a mobile phase of 16 ml ethyl acetate: 4 ml methanol: 3 ml of concentrated ammonia solution. The plates were viewed under UV lamp at 254 nm. Movement of compounds present in different brands was recorded as retention factors (RF values) on TLC plates.

For detection of artesunate (B1 & B2), artesunate tablets 60 mg was extracted with 12 ml of methanol (5mg/ml). The development phase for artesunate was 18 ml of ethyl acetate, 4 ml of acetone and 0.1 ml of glacial acetic acid. The plates were checked under UV 254 nm firstly and then stained with sulphuric acid for detection of artesunate compared with reference standard.

For quality assurance of dihydroartemisinin 40 mg and piperaquine phosphate 320 mg fixed dose combination tablets (C1 & C2), these combination tablets were dissolved in 20 ml

of methanol (2 mg/ml of dihydroartemisinin & 16 mg/ml of piperaquine). The development phase was 16 ml ethyl acetate: 4 ml methanol: 3 ml of concentrated ammonia solution. Firstly, the presence of piperaquine was detected under 254 nm. For the detection of dihydroartemisinin, the chromatoplate was exposed to sulphuric acid staining and compared with reference standard.

For assessment of artemether 20 mg and lumefantrine 120 mg fixed dose combination tablets (D1 & D2), these combination tablets were dissolved in 10 ml of acetone (2 mg/ml of artemether & 12 mg/ml of lumefantrine). The development phase was 4 ml of ethyl acetate, 2 ml of glacial acetic acid and 18 ml of toluene. The plate was detected under UV 254 nm for detection of lumefantrine firstly. For the detection of artemether, the chromatoplate was exposed to sulphuric acid staining.

3. Results

Two different brands of mefloquine 250 mg, two brands of artesunate 60 mg, two brands of dihydroartemisinin 40 mg and piperaquine phosphate 320 mg fixed dose combination tablets, two brands of artemether 20 mg and lumefantrine 120 mg fixed dose combination tablets imported brands and local manufactured in Myanmar were analysed in this study. All samples were within shelf life at the time of the study. They were all registered Myanmar food and drugs administration.

The visual inspection parameters like uniformity of shape, uniformity of color, presence of physical damage (cracks, breaks, abrasion, sticky), other observations (foreign contaminants, dirty marks, proper seal) and labeling requirements were passed in all brands of different anti-malarial drugs. For assessment of the simple disintegration test, results of all brands showed less than 30 minutes of the GPHF range.

For the weight variation test, the British Pharmacopoeia specifies that not more than two of the individual weights should deviate from the average weight by more than 5%, and none should deviate by more than 10%⁸. From Table 2, it is observed that all brands of different anti-malarial drugs were within the British Pharmacopoeia standard range. All brands 100% passed the weight variation test. This test is important especially where the drug substance forms the greater part of the tablet mass as dosage is obviously linked with tablet weight, and a compliance with this standard helps to ensure that uniformity of dosage is achieved. The inter batch weights differences could be attributed to the variations in percentage of excipients especially diluents or bulking agents, which is usually the decision of the formulation pharmacist.

Thin Layer Chromatography is of importance for the verification and identification of medical products. It is also used to decide on the presence or absence of impurities or degradation products. From Table 2, the retention factor (RF value) for reference standard was 0.466. All samples were within the range of the RF value ± 0.1 (British Pharmacopoeia range). By using the GPHF minilab guidelines, all different brands of anti-malarial drugs including A1, A2 for mefloquine, B1, B2 for artesunate, C1, C2 for dihydroartemisinin and piperaquine fixed dose combination tablets and D1, D2 for artemether and lumefantrine combination tablets representing 100% of thin layer chromatography. The different brands of anti-malarial drugs assessed passed all the parameters of visual inspection, labeling inspection, weight variation tests, simple disintegration tests, present of active ingredient and absence of contamination spots.

Table (1) Labeling Description of Different Anti-malarial Drugs

Code	Manufacture Date	Expiry Date	Batch number	Myanmar FDA Registration number
A1 (Mefloquine)	14/3/2014	14/3/2017	0128146	R1609A4998
A2 (Mefloquine)	01/2015	08/2018	0920	R1708AA007
B1 (Artesunate)	07/2014	07/2017	130733	R1709AA007
B2 (Artesunate)	02/2014	02/2017	140281	R1502A3761
C1 (DHA+Piperaquine)	08/2014	07/2017	155042	R1608A8950
C2 (DHA+Piperaquine)	19/6/2014	19/6/2017	0505	R1409A3679
D1 (Artemether+Lumefantrine)	06/2014	05/2016	FA193	R1504A3902
D1 (Artemether+Lumefantrine)	08/2014	07/2016	140201	R1603AA3038

Table (2) Physicochemical Characteristics Assessment

Code	Disintegration Time (min)	Mean Tablet Weight (No. of tablets outside range)	Retention Factor (Sample error in %) Reference standard=0.466
A1 (Mefloquine)	6.5	307.80 ± 15.39 (0)	0.459(1.5%)
A2 (Mefloquine)	6.0	332.54 ± 16.63 (0)	0.452(3%)
B1 (Artesunate)	4.5	76.45 ± 3.82 (0)	0.460(1.4%)
B2 (Artesunate)	6.5	82.62 ± 4.13 (0)	0.466(0%)
C1 (DHA+Piperaquine)	4.5	414.70 ± 20.74 (0)	0.466(0%)
C2 (DHA+Piperaquine)	5.5	424.32 ± 21.22 (0)	0.459(1.5%)
D1 (Artemether+Lumefantrine)	4.0	185.55 ± 9.28 (0)	0.459(1.5%)
D1 (Artemether+Lumefantrine)	5.5	190.22 ± 9.51 (0)	0.459(1.5%)

4. Discussion

The quality of commercially available drugs varies greatly among countries. Due to lack of regulations and poor quality control practices in some countries, the amount of the active ingredient can be inconsistent. Poor formulation techniques can affect the release of active ingredients from a tablet, with some tablets releasing very little amount of drug. Some drugs may be contaminated with other substances. Poor storage conditions, especially in warm and humid tropical environments may contribute to chemical degradation of many pharmaceuticals⁹.

Visual inspection of a drug and its packaging is usually an initial indication of the genuineness of the medicament. Labeling is also a critical parameter as the patients' safety may be dependent on the label of the medication. Other labeling requirements of expiry date, batch number and regulatory authority registration number are required¹⁰.

Tablets are required to be sufficiently hard to withstand handling without crumbling or breaking and also sufficiently soft for easy disintegration in the stomach or intestine in order to make the drug available to the body. In this study, the simple disintegration test recommended by the GPHF for mainly for on-the-field assessment and resource poor setting is adopted. It requires that uncoated tablets disintegrate within 30 minutes³.

Two different brands of mefloquine 250 mg, two brands of artesunate 60 mg, two brands of dihydroartemisinin 40 mg and piperaquine phosphate 320 mg fixed dose combination tablets, two brands of artemether 20 mg and lumefantrine 120 mg fixed dose combination tablets were assessed using the quality control parameters of visual inspection, weight uniformity test, simple disintegration tests and thin layer chromatography profile. The results indicated that 100% of all different brands of anti-malarial drugs passed all quality parameters of GPHF minilab. And all the brands complied with falling within the RF range in comparison with their reference standards.

In the present study we have made an attempt to know the qualitative difference in the different brands of the anti-malarial drugs. These drugs are also manufactured and marketed by innumerable companies which include both multinational and local companies. The study is simple and only compares the different parameters of different brands of anti-malarial drugs with their standards. It can be easily carried out in a simple set up. The study can be improved upon by including quantitative analysis of the drug but this needs sophisticated equipment for the study. The study has attempted to compare the qualitative difference in the different brands of anti-malarial drugs, but actual concentration of the drug needs to be evaluated. The study finds to be simple and is useful especially for monitoring the quality of the drugs available in the local market. This study also can be used as initial vital tool to access the quality of the drugs¹¹.

5. Conclusion

Thin layer chromatography (TLC) method could verify the drug's identity and quality rapidly and thus detect fake medicines by employing inexpensive analytical techniques. Standard quality control parameters of GPHF minilab always should be monitored to check counterfeits and poor-quality of medicines in Myanmar not only for anti-malarial drugs but also for other kinds of medicine for getting standard drug quality.

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Drug utilization of Non-Steroidal Anti Inflammatory Drugs in No. (2) Defence Services General Hospital (1000 Bedded)

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Abstract- Non steroidal anti-inflammatory drugs (NSAIDs) are the most commonly used drugs for years for the management of pain and inflammation with good efficacy and represent most widely prescribed class of medications and are used as over the-counter-drugs in the world. Drug utilization studies are useful elucidation tools for prescribing habits in particular therapeutic field and they play a pivotal role in helping the healthcare system to understand, interpret and improve the prescribing, administration and use of medications. This study was conducted to find out the drug utilization patterns of NSAIDs in No (2) Defence Services General Hospital (1000 Bedded) out of medical records compiled from July to September, 2014. This was a hospital-based, cross-sectional, descriptive study using the World Health Organization Anatomical Therapeutic Chemical classification (ATC)/Defined Daily Dose (DDD) methodology and Drug Utilization (DU) 90% index. Among the 1147 admitted patients, 412 patients (36%) were prescribed NSAIDs. Total utilization of NSAIDs was 11.89 DDD/ 1000 inhabitants per day. Paracetamol was the most used drug, followed by diclofenac, ketorolac, celecoxib and ibuprofen was the least used drug. NSAIDs were prescribed for fever 173 (42%), followed by post operative pain 108 (27%) and low back pain 38 (9%) patients. Three drugs were included in drug utilization 90% segment. Gastro protective agents were prescribed in 107 (26%) patients along with NSAIDs. Non selective NSAIDs were prescribed in 401 (97%) patients and selective NSAIDs were in 11 (3%) patients. The average number of NSAIDs per patient was 1.3. These data can provide useful information to improve prescribing patterns of NSAIDs.

Keywords: NSAIDs, selective NSAIDs; Drug utilization studies; ATC/DDD methodology; gastro protective agents; drug utilization 90% segment

1. Introduction

Drug utilization studies have been interested since early 1960¹. Drug utilization research was defined by World Health Organization (WHO) in 1977 as the marketing, distribution, prescription, and use of drugs in a society, with special emphasis on the resulting medical, social and economic consequences².

The Anatomical Therapeutic Chemical (ATC) classification system³ and new unit of measurement the defined daily dose (DDD)^{4,5} were developed to measure drug use.

In the ATC classification system, drugs are divided into different groups according to the organ or system on which they act and according to their chemical, pharmacological and therapeutic groups at five levels. Defined daily dose (DDD) is the assumed average maintenance dose per day for a drug used on its main indication in adults. The DDD is a unit of measurement; it is not a recommended dose and may not be a real dose³.

The Drug Utilization 90% (DU90%) index was introduced as a simple, inexpensive and flexible method for assessing the quality of drug prescriptions. It identifies the drugs accounting for 90% of the volume of prescribed drugs after ranking the drugs used by volume of DDD^{6,7}.

NSAIDs are the most commonly used drugs for years for the management of pain and inflammation with good efficacy and represent most widely prescribed class of medications in the world and are used as over the counter drugs^{8,9}. Despite wide clinical use of classical NSAIDs, their gastro-intestinal toxicity is a major clinical limitation. This adverse effect is associated with their ability to inhibit COX-1 in the gastrointestinal tract. NSAIDs are described as leading cause of adverse drug reactions¹⁰. Subsequently, the selective COX-2 inhibitors emerged as potentially gastro-friendly NSAIDs and it was conceptualized that

sufficient therapeutic benefits are achieved by selective COX-2 inhibition^{11, 12}.

At first glance these COX-2 inhibitors look like solution to NSAIDs related GI complications. However, post marketing surveillances unmasked various adverse cardiovascular effects¹³. Therefore, the uses of COX-2 selective inhibitors have created a sense of insecurity not only among prescribers but also among consumers. Moreover with variety of NSAIDs that are presently available, it is difficult at times to select a particular NSAID on a rationale basis alone but on empiricism. Thus it is needed to improve the safe, appropriate and effective prescribing of NSAIDs.

For the developing country like Myanmar, a national drug policy is needed to improve or rationalize drug use. To achieve this, it is very important to determine drug use patterns and monitor drug use profiles over time. Without knowledge of how drugs are being prescribed and used, it is difficult to suggest measures, to improve prescribing habits or to initiate a discussion on rational drug use. Information on the past performance of prescribers is the linchpin of any auditing system¹⁴. Therefore drug utilization study of NSAIDs is more interested.

2. Materials and Methods

This study was hospital-based, cross-sectional (descriptive) study conducted at No (2) Defence Services General Hospital (1000 Bedded). Data were collected from medical records compiled from July to September, 2014. Patient details, including age, sex, address and length of hospital stay, were recorded. Each drug prescribed was recorded including its dosage form, route of administration, frequency of administration, indications for use, and duration of therapy. The collected data were entered to the preformed format for analyzing of data.

Drugs utilization data were analyzed by using the WHO ATC/DDD methodology³. The ATC codes for each NSAID were obtained from ATC index with DDDs 2012. From this, DDD of each drug was obtained.

Then the total numbers of DDDs and DDD/ 1000 inhabitants/day for each NSAID were calculated from the collecting data by the following formulas.

$$\text{Drug usages (DDDs)} = \frac{\text{Items issued (tablets or ampoules etc.)} \times \text{Amount of drug per item}}{\text{DDD (from ATC index with DDDs 2012)}}$$

$$\text{DDD/1000inhabitants/day} = \frac{\text{Total consumption in DDDs} \times 1000}{\text{Covered inhabitants} \times \text{Days in the period of data collection}}$$

3. Results

Table (1) Prevalence of NSAIDs uses

Category	Number of patients	Percentage
Patients who are prescribed NSAIDs	412	36
Patients who are not prescribed NSAIDs	735	64
Total	1147	100

Table (2) Disease patterns of the patients who were prescribed NSAIDs

Diseases	No. of patients	Percentages
Low Back Pain	38	9
Surgical wound pain	108	27
Fever	173	42
Other Muscle pain	93	22
Total	412	100

Table (3) Number of NSAIDs Prescribed Per Patient

Total no. of NSAIDs	538
Total no. of patient	1147
Average no. of NSAIDs per patient	0.47

In this study, **one NSAID** represents one generic name of any NSAIDs given irrespective of its trade name, amount, duration, frequency and route.

Table (4) Drug Utilization 90% Segment of NSAIDs

Sr no.	NSAIDs	ATC Code	Total DDD	DDD/1000 Inhabitants/day	Percentage
1	*Paracetamol	N02BE01	517.167	5.01	42.19
2	*Diclofenac	M01AB05	485.5	4.69	39.41
3	*Ketorolac	M01AB15	150	1.45	12.18
4	Celecoxib	M01AH01	61.6	0.59	4.96
5	Ibuprofen	M01AE01	15	0.15	1.26
	Total		1229.267	11.89	100

*Drug Utilization 90 % Segment Drugs (n= 3 Drugs)

4. Discussion

In the present study 36% of patients were prescribed NSAIDs. This is less than previous studies¹⁵ 87% in Myanmar. This may be due to the previous study hospital was orthopaedic hospital where NSAIDs were widely used. But present study was general hospital.

Demographic characteristics showed that percentage of males using NSAIDs was more than females. In the study hospital NSAIDs were commonly prescribed for reducing fever and post-operative pain.

In the present study, it is well observed that most of the patients were prescribed non selective NSAIDs for treatment of various pain conditions and inflammatory states. It may be for the reason that although non selective NSAIDs were associated with gastrointestinal complications, they have low cardiovascular risks than that of selective NSAIDs¹⁶.

Drug consumption data were expressed as defined daily doses (DDD) per 1000 inhabitants per day. The highest value of 5.01 DDD /1000 inhabitants/day was accounted for paracetamol indicating that it was the popular drug of choice for pain and fever, followed by diclofenac with the value of 4.69 DDD /1000 inhabitants/day.

In the present study only three drugs were included in DU 90% segment. Paracetamol was top in this. The DU 90% prescribing indicator is useful to demonstrate how quickly drug/s penetrated the market¹⁷. In one study, meloxicam entered into the DU 90% soon after its launch on the market (2002), and its use increased constantly over the years accounting for 3% in 2002 and 25% in 2005 and 2006¹⁷.

Paracetamol was the most prescribed NSAIDs in the present study. Moreover, in all combination therapies paracetamol was included. In the previous study¹⁵, paracetamol was the third most prescribed NSAIDs. It was stated that at therapeutic doses, paracetamol is considered safer than other anti-inflammatory drugs and it is much cheaper than non-selective NSAIDs and COX-2 selective inhibitors¹⁸. For elderly suffering from musculoskeletal conditions paracetamol is also much safer option in clinical use¹⁸. So it is well observed that paracetamol should be prescribed in most pain condition and reducing fever. So in the present study paracetamol was the most prescribed drug,

In the present study, paracetamol and diclofenac combination therapies were used in 30 patients (7%). This may be due to the acetaminophen's action is inadequate for more severe pain after dental surgery and has demonstrated a ceiling effect at 1,000 mg¹⁹. Moreover, it was found that acetaminophen's relatively short acting analgesia limits its usefulness as a mono-therapy for the treatment of moderate to severe postoperative pain²⁰.

Diclofenac was the second most used NSAID in the current study. It was commonly used in other studies as analgesic drug^{15, 21, 22, 23}. Diclofenac has analgesic, antipyretic, and anti-inflammatory activities. Its potency against COX-2 is substantially greater than that of indomethacin, naproxen, or several other non-selective NSAIDs. The incidence of serious gastrointestinal adverse effects did not differ between celecoxib and diclofenac²⁴. Therefore diclofenac was widely used in current study hospital.

Ketorolac was commonly used in post operated pain cases in the present study. Ketorolac has been used alternative to opioids for the treatment of moderate to severe pain and is administered intramuscularly, intravenously, or orally. Unlike opioids, tolerance, withdrawal, and respiratory depression do not occur²⁵.

Celecoxib and ibuprofen were not widely used in the present study. This was similar to previous study¹⁵. In the present study concomitant gastro protective agents were not widely used along with NSAIDs.

In the current study average number of NSAIDs per patient was 1.3. This means more than one NSAID was prescribed in one patient when they he/she was prescribed NSAIDs. Average number of drugs per person is an important index of prescription audit. It is preferable to keep the mean number of drugs per prescription as low as possible, since higher figures always lead to increased risk of drug interaction and unwanted side effects²⁶.

In the present study there was only five NSAIDs were used. This documentary evidence can give some help to inform clinicians, researchers, and/or policymakers.

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CASE REPORT

NEUROCYSTICERCOSIS

Zin Phy Hlaing, Kaung Htut, Thet Naing Win, Thant Lwyn San & Tin Moe Mya

Case summary

Twenty-four years old male officer was admitted to DSGH (1/1000 Bedded) at 2.6.2016 for chief complaint of generalized fits off and on for 4 times within 3 months duration. Fits were tonic, and clonic in nature. There was no history of neurological deficits after attacks. Accompanying symptoms such as projectile vomiting and fever were not present. But history of tingling and numbness sensation of left upper limb were found within 3 months durations. There was no past history of head injury.

On examination: general condition was fair. No abnormalities were detected on respiratory and cardiovascular system. Abdominal examination revealed normal. Sensory and motor deficits were not found. Ultrasound abdomen showed no abnormalities was detected. Lumbar puncture was done under aseptic condition and CSF appeared normal color and no tension. CSF was sent for Gram stain and ZN stain. But organism was not found and CSF Culture was sterile. CT (Head) (NECT & CECT) was done and solitary ring enhancing lesion was detected in right parietal region measuring 1x1.2x1.3 cm in diameter with perilesional oedema. MRI (NEMR & CEMR) examination of the brain was done at 14.6.2016 and solitary cystic ring enhancing SOL at right parietal region was detected. Radiological opinion was favorable of neurocysticercosis and differential diagnosis was cystic tuberculoma.

Operation was done under general anesthesia at 16.6.2016 by Consultant Neurosurgeon Lt Col Thant Lwyn San. Round to oval shaped cyst measuring 1x1.2x1.3 cm was noted at right parietal lobe. Cyst wall was thickened and cyst was containing whitish turbid fluid. Cyst was removed and then sent for histological examination.

Histological examination of tissue showed cyst which was convoluted, and degenerated and cyst was filled with hyalinized materials. Cyst wall was infiltrated by intense inflammatory cells such as lymphocyte, plasma cells, eosinophils and multinucleated giant cells. Fibrosis, gliosis and necrosis were found in surrounding areas. There were no granuloma formation and no features suggestive of tuberculosis. Diagnosis was compatible with neurocysticercosis (right parietal region).

On 7th Post-operative day, stitches were out and patient had recovery and discharged from hospital. There was no more epilepsy like fit attack.

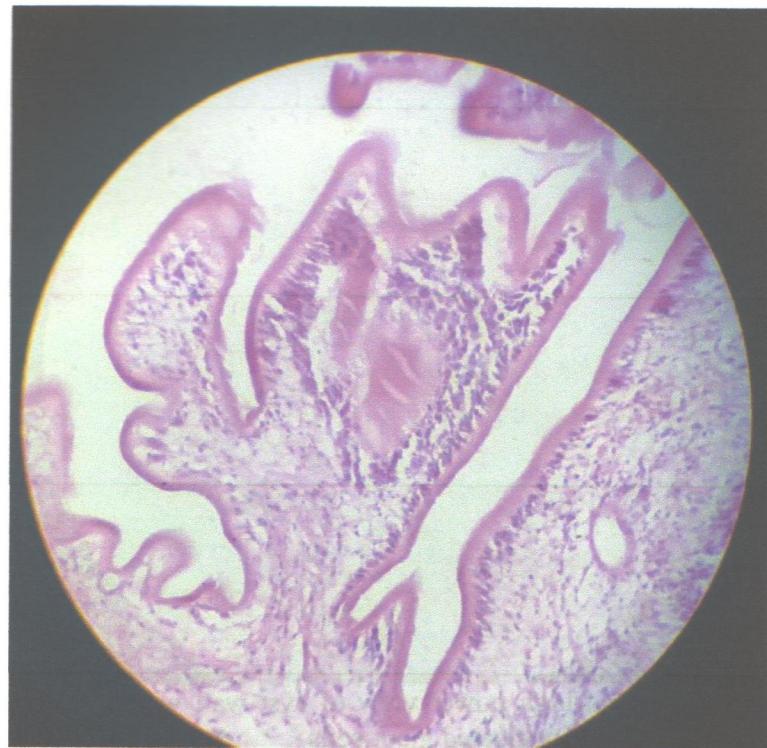
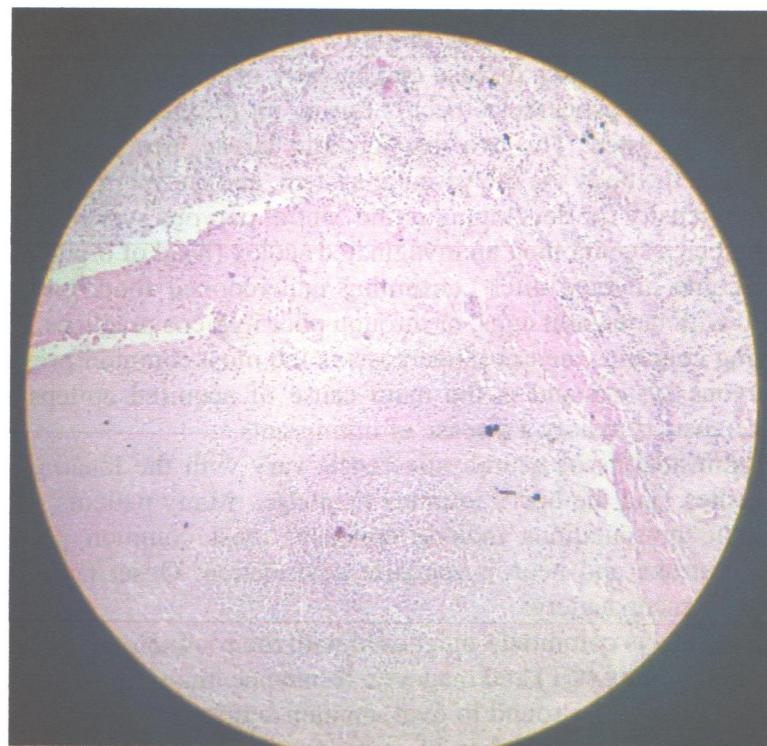


Figure 1. Convolved cyst and cyst wall



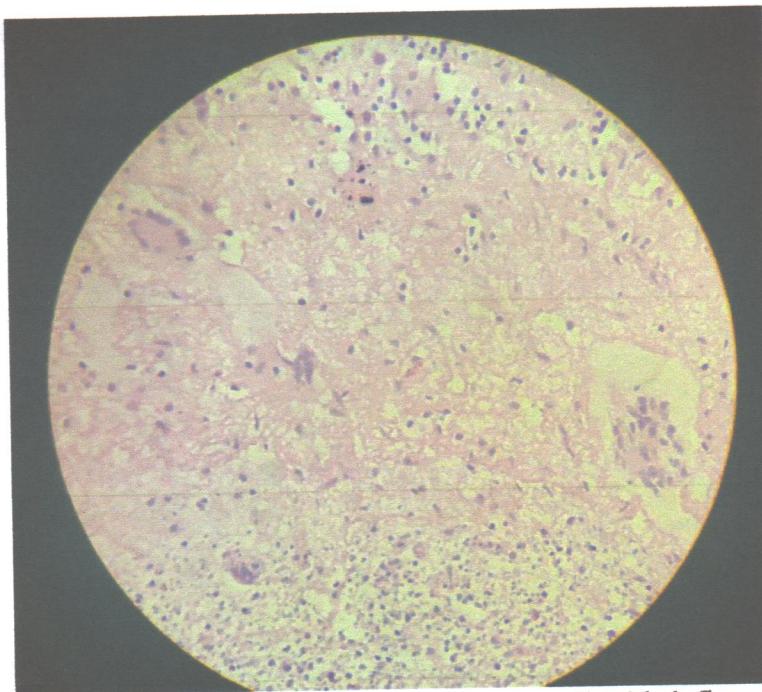


Figure 2&3. Low power and high power view of cyst wall with inflammatory infiltrates, necrosis and multinucleated giant cells

Literature review

Globally, cysticercosis is the most common parasitic infection involving the brain. *Taenia solium*, a tapeworm, causes disease endemically in Mexico, Central America, South America, Asia and Africa. Cysticercosis occurs during an infection by the larval stage of *T. solium* when ova are ingested. The ova develop into larvae, penetrate the intestinal wall, disseminate throughout the body via the vascular system, and encyst in the tissue as cysterci. Cysterci have a propensity for developing in the central nervous system (60% of cases) and are classically described as containing an invaginated scolex (head of organism).

Humans become infected after consuming undercooked food, particularly pork, or water contaminated with tapeworm eggs, or through poor hygiene practices.

In developing countries, neurocysticercosis is the most common preventable parasitic disease of the nervous system and is the main cause of acquired epilepsy. In the United States, neurocysticercosis is mainly a disease of immigrants.

Clinical manifestations of neurocysticercosis vary with the locations of the lesions, the number of parasites, and the host's immune response. Many patients are asymptomatic. Possible symptomatic presentations include epilepsy: most common presentation (70%), headache, dizziness, stroke and neuropsychiatric dysfunction. Onset is usually subacute to chronic, but seizures present acutely.

Neurocysticercosis is commonly diagnosed with the routine use of diagnostic methods such as computed tomography (CT) and magnetic resonance imaging (MRI) of the brain.

Identifiable cysterci are round to oval, contain semitranslucent to whitish fluid, and usually measure 1 to 2 cm. The number of cysts can range from one to several hundred. Cysterci are most commonly located in the cerebrum (91%) but can form in the ventricles (6%) and the subarachnoid space (2%). Spinal cord involvement is rare, accounting for only 0.2% of cases. Other nonspecific neurocysticercosis findings include cerebral edema and compression.

The racemose form of neurocysticercosis consists of a single large vesicle or mass of vesicles that often resembles a cluster of grapes and may measure up to 10 cm. This expansive type of neurocysticercosis accounts for 10% of cases and is most frequently found in the basal cisterns.

Histologically, cysticerci manifests 4 distinct stages. The vesicular stage consists of vesicles with viable organisms. Each viable organism is composed of a larva containing an invaginated scolex (head) and surrounded by translucent fluid that is lined by a thin membranous wall.

In the colloidal stage, the vesicle fluid becomes more turbid and the larvae become hyalinized. The inflammatory infiltrate becomes more intense, includes lymphocytes, some neutrophils and eosinophils, and the formation of multinucleated histiocytic giant cells.

The granular-nodular stage involves progressive degenerative changes with increased larval decay, vesicle involution, and thickening of the vesicle wall. The larvae in this stage begin to mineralize with calcium. The degenerative process ends with the nodular-calcified stage. In this stage, cysticerci are replaced by collagen and calcified.

Neurocysticercosis was treated with anthelmintic medications like praziquantel and albendazole. The prognosis of neurocysticercosis is multifactorial and ultimately depends on the host immune response, disease duration, and parasite location, load, size and stage.

Conclusion

This case was presenting with adult type epilepsy. After thorough examination with CT, MRI brain, think about differential diagnosis of neurocysticercosis and cystic tuberculoma.

But final diagnosis was confirmed by histological examination. In this case, solitary cyst was located in superficial right parietal region, so patient was lucky and easy to remove completely.

Therefore the patient was totally free from his symptom and get well-being after operation. Neurocysticercosis is the common helminthic disease of the nervous system. Massive cysticercosis of the brain causes convulsions and death, and in the heart may cause arrhythmias and sudden death. Cysticerci in the retina may lead to blindness. So health personnel must be aware of cysticercosis which may cause fatal to individual who consuming undercooked pork.

References

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