MYANMAR
HEALTH SCIENCES
RESEARCH JOURNAL

DEPARTMENT OF MEDICAL RESEARCH
(Lower Myanmar)
No. 5, Ziwaka Road
Dagon Township
Yangon 11191, Myanmar
Published since 1989

Volume 22 Number 1 April 2010

CONTENTS

Genetic population structure of *Aedes aegypti* mosquitoes at various spatial scales in Myanmar..... 1
Thaung Hlaing, W. Tun Lin, Pe Than Htun, Sein Min, Sein Thaung & Catherine Walton

Cloning of the hepatitis B surface antigen gene (3): integration of the recombinant HBsAg gene containing plasmid into genomic DNA of *Pichia pastoris* yeast cells….. 8
Win Aung, Yeon-Hee Kim, Hyunah Ki , Suk-Hoon Ha & Khin Pyone Kyi

Erythrocyte antioxidant enzymes activities and plasma carbonyl protein levels in smokers..... 14
Khin Than Yee, Kyu Kyu Maung, Myo Win, Theingi Myint, Kyaw Oo & Zaw Myint

Detection of brucellosis in animal handlers and raw milk of selected dairy cattle farms in Yangon..... 20
Thuzar Myint, Win Maw Tun, Wah Wah Aung, Zin Mar Aung, Myat Thida, Aye Aye Maw, Aye Yin Shwe & Khin Nwe Oo …….

Patterns of anti-tuberculosis drug resistance among HIV patients with pulmonary tuberculosis attending the Specialist Hospital, Waibargi, Yangon……... 25
Wah Wah Aung, Sabai Phyu, Htin Aung Saw, Ti Ti, Phyu Win Ei & Rai Mra

Knowledge, attitude, and prevention practice on avian influenza among housewives in Mingalar Taungnyunt Township, Yangon……..... 32
Yae Chan, Han Win, Aung Thu, Sandar Kyi, Kyu Kyu San, Myat Myat Thu, Ni Ni Aye, Khin Moe Latt & Khin Myat Tun

Service factors related to defaulting TB treatment in Myanmar..... 39
Tin Mi Mi Khaing, Thin Thin Yee, Tin Maung Swe, Myat Myat Moe, Saw Saw, Si Thu Aung, Win Maung & Aye Htun

Immunohistochemical study of human papillomavirus infection in squamous cell carcinoma of oral cavity……..... 46
Thar Htet San, Yi Yi Myint, Wai Wai Khaing , Sann Sanda Khin, Phyu Synn Oo, Myo Htet Htet Khaing, Khin Thi Lwin & Htun Naing Oo

Prevalence of hypertension and its associated risk factors in Pintaya Township, Southern Shan State…….. 51
Khin Myat Tun, Han Win, Khin Thet Wai, Theingi Thwin, Zaw Myint, Tin Oo, Moh Moh Win, Moe Thida Kyaw & Maung Maung Myint

Social burden of malaria on family in Hlegu Township, malaria endemic area……... 58
Le Le Win, San Shwe, Than Win, Thandar Min & San San Aye

Ineffectiveness of a single dose of rifampicin, ofloxacin and minocycline (ROM) chemoprophylaxis in preventing leprosy…….. 62
Khin Nwe Oo, Khin Thet Wai, Kyaw Myint & Mg Mg Gyi

Short Report: Hepatitis B vaccination coverage among trainees in Central Institute of Civil Services (182nd batch, 2009), Phaung Gyi ……. 67
Min Min Win, Aung Zaw Latt & Win Aung

……

Editor-in-Chief

Dr. Myo Khin

Editor

Dr. Ye Htut

Associate Editors

U Aung Myo Min Dr. Ni Thet Oo

Editorial Board

Prof. Dr. Pe Thet Khin
Prof. Dr. Chit Soe
Prof. Dr. Mg Mg Khin
Prof. Dr. Yin Yin Soe
Prof. Dr. Myat Thandar
Prof. Dr. Kyi Kyi Thinn
Prof. Dr. Myo Win

Prof. Dr. Myo Oo
Dr. Khin Nwe Oo
Dr. Thaw Zin
Dr. San Shwe
Dr. Khin May Oo
Dr. Win Aung

Business Manager
Dr. Ye Tint Lwin

Editorial Manager
U Htain Win

Production Manager

U Ye Thway
Daw Win Win San

Restricted for Internal Use Only

The Myanmar Health Sciences Research Journal, Vol. 22, No. 1, 2010

Genetic population structure of *Aedes aegypti* mosquitoes at various spatial scales in Myanmar

Thaung Hlaing, **W. Tun Lin, *Pe Than Htun, *Sein Min, *Sein Thaung &*Catherine Walton*

*Medical Entomology Research Division
**Department of Medical Research (Lower Myanmar)
***University of Manchester, UK

Dengue fever/dengue haemorrhagic fever (DF/DHF), the most common mosquito-borne viral disease, is a re-emerging public health problem in the world particularly in the tropics. At present, dengue vector control still depends on the selected use of certain insecticides in targeted dengue patient areas particularly during outbreaks in disease endemic countries including Myanmar. An understanding of genetic population structure and dispersal ability in dengue vector *Aedes aegypti* populations may provide useful information for effective vector control strategies. It is useful to understand the genetic variability of local mosquito population for the development of current vector control purposes. The study therefore has attempted to infer the genetic population structure and the extent of dispersal ability of *Aedes aegypti* at various spatial scales using different statistical models. Based on 13 microsatellite loci data, a significantly low level of genetic structuring was found at all spatial scales among three clusters of mosquito populations. Average pairwise F_{ST} values ranged from 0.026 within 5 km to 0.043 across >500 km distance. Landscape genetics results showed different genetic heterogeniety among and within regional clusters. The findings of restricted gene flow and small scale population structure support the nature of limited dispersal ability of *Aedes aegypti* and possible effect of different insecticide usages on local mosquito populations. On the other hand, passive mosquito dispersal which is a problem for dengue vector control also plays an important role in shaping large scale genetic structure.

INTRODUCTION

Dengue/dengue haemorrhagic fever (DF/ DHF) is one of the major re-emerging public health problems in the world, particularly in the tropics and subtropics with an estimated 50-100 million people infected annually which can lead to hospitalisation in half a million of severe cases [1]. Dengue virus is mainly transmitted to humans through the bite of *Aedes aegypti* mosquitoes. Southeast Asia (SEA) including Myanmar has experienced severe dengue epidemics since the 1950s, particularly in urban areas [2-4]. The first DHF case in Myanmar was reported in 1970 in Yangon [5]. In the following decades, dengue outbreaks occurred in 1987, 1991, 1994, 1998 and the largest in 2001 with 15,361 reported cases of DHF/DSS, including 192 deaths [6]. The spread of dengue viruses and *Aedes aegypti* in SEA was mainly via trade and transport between populations by shipping [7, 8]. Because it is a highly domestic species that feeds on humans and breeds in and around human habitation, *Aedes aegypti* and the disease it transmits have proliferated along with human population growth, economic development, increased mobility and uncontrolled urbanisation.

At present, the reduction of dengue transmission relies on appropriate vector control as vaccine development is still underway [9]. It is useful to understand the genetic variability of local mosquito population for the development of current vector control purposes. Genetic population structure usually results from a combination of several contemporary and historical processes such as dispersal ability, mating patterns, environmental barriers and demographic history [10]. Since we know relatively little about the population history of *Aedes aegypti*, the study has attempted to infer the genetic population structure and the extent of dispersal ability of *Aedes aegypti* at various spatial scales using different statistical models.

Microsatellite markers have been recently used for determining genetic population structure in *Aedes aegypti*. Most of these small scale studies generally conclude low levels of genetic differentiation [11-14]. We do not have any statistics on genetic population structure of *Aedes aegypti* in Myanmar to date. The aims of this study are therefore to infer the genetic population structure of *Aedes aegypti* at a hierarchy of spatial scales across mainland Myanmar and to determine the factors shaping genetic structure at each scale. In addition to using conventional population-based methods we also apply landscape genetics approaches [15]. The findings are interpreted for their significance for vector control in Myanmar and for the future utility of landscape genetics to identify the factors shaping the genetic structure of this species on a wider scale.

MATERIALS AND METHODS

Mosquito sampling

Aedes aegypti mosquito larvae from natural populations were sampled at a hierarchy of spatial scales (5 km, 50 km and 500 km) during 2004-2005. The sampling strategy, which is fundamental in population genetics, was carefully designed and arranged in clusters to optimise a range of spatial scales over an extensive geographical area representing different ecological regions. There were three main collection regions (clusters): Yangon, Meiktila and Myitkyina, each of which comprised four collection sites, three that were ~5 km apart and a fourth that was ~50 km apart. A site covered an area of ~500m in diameter as a reasonable estimate of neighbourhood size based on the flight range of *Aedes aegypti* [16, 17].

Within this area third and fourth stage larvae and pupae were collected from 50 different water storage containers (such as tanks, indoor cisterns, tyres etc.) in and around the houses. Larvae and the adults (which hatched from the collected pupae) were morphologically identified using standard taxonomic keys. Identified larvae were preserved in 95% ethanol and adults were placed in silica gel. The locations of the sampled containers were recorded using a global positioning system (GPS). For microsatellite genotyping a single individual was selected at random from each container to avoid incidental sampling of close relatives.

DNA extraction and microsatellite genotyping

DNA was extracted from individual mosquitoes using a standard phenol/ chloroform method [18]. Thirteen microsatellite loci which have been characterised and found to have suitable levels of variation in *Aedes aegypti* [19] were amplified in two sets of multiplex PCR. Each reaction comprise of dried DNA template (1 µl of a 1:400 dilution); 1 µl of Primer Mix (containing 0.2 mM of each primer with the forward primer of each pair fluorescently labelled with HEX, FAM or NED); and 1 µl of Qiagen Master Mix (QIAGEN). The reaction conditions were an initial denaturation step at 95˚C for 15 mins; 35 cycles at 94˚C for 30 sec, 55˚C for 90 sec and 72˚C for 90 sec; and a final extension of 10 mins at 72˚C. The amplified products were run on an ABI 3730 capillary sequencer (Applied Biosystems Inc.) and were genotyped using the GeneMapper software 3.7 (Applied Biosystems Inc.).

Genetic analyses

Allelic richness (R_S), observed and expected heterozygosity (H_O and H_E) were estimated for each locus in each population using ARLEQUIN 3.01 [20]. Genetic differentiation between pairs of populations was also estimated using F_{ST} as a distance matrix. Significance was estimated at the 5% level by 1,000 permutations of the genotypes among populations. Mantel test was implemented to test for isolation by distance using $F_{ST}/(1- F_{ST})$ as an estimate of pairwise genetic distances between populations [21].

A Bayesian clustering method, TESS 2.0 [22], was used to estimate genetic clusters. It is based on the assumption that spatially close individuals are likely to be genetically related. For each value of clusters (k) from 2 to 12, five replicates of 10 independent MCMC chains each with 12,000 sweeps and a burn-in period of 2,000 sweeps was run. The optimal number of k can be determined from the runs with the highest posterior likelihoods. The populations were tested for population bottleneck using the intra-locus k-test and inter-locus g-test [23]. Both tests were conducted using the Excel Macro KGTESTS [24].

RESULTS

Variation in 13 microsatellite loci for 304 individuals from three clusters was analysed. All loci were polymorphic in all populations. Average allelic richness (R_S) were 5.6, 5.42 and 5.38 for Yangon, Meiktila and Myitkyina populations, respectively. Average observed heterozygosity (H_O) (0.065) and average gene diversity over loci (0.73) were more or less the same in all populations. The F_{ST}-based estimates for all pairs of populations revealed that even sites that were only ~5 km apart were significantly genetically differentiated although the level of differentiation was low (average F_{ST} value of 0.026) (Table 1). It was possible that there were some false positives but these would have minimal effect on overall findings since the majority of tests were positive (62 out of 66, P≤0.0001).

Table 1. Pairwise population differentiation of *Aedes aegypti* mosquitoes from Myanmar

	Within clusters		Between clusters	
	~ 5 km	~ 50 km	~ 500 km	>500 km
Average pairwise F_{ST}	0.026	0.032	0.039	0.043
Range	0.001 - 0.039	0.013 - 0.045	0.029 - 0.088	0.021 - 0.083
Significant pairwise	8/9	9/9	30/32	15/16

As spatial scale increased, the average F_{ST} value and level of significance also increased. This was consistent with the signal of isolation by distance found in the populations (Fig. 1). The Mantel test showed that genetic and geographic distances were significantly correlated although the level of correlation was low ($R^2 = 0.07$; P = 0.041).

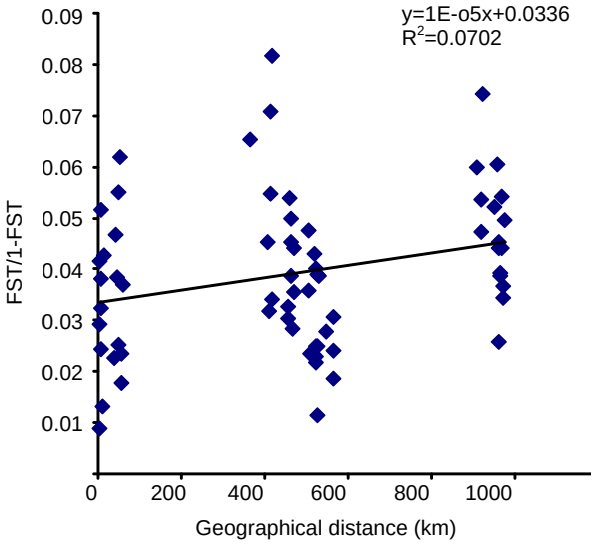


Fig 1. Correlations of genetic and geographic distance for 12 populations of *Aedes aegypti* in Myanmar

When TESS was run with the spatial interaction parameter (ψ) set to 0, for k=2-12, all the populations were always mixed. With ψ set to 0.6, the likelihood of TESS increased with increase in k, reflecting the low but significant differentiation

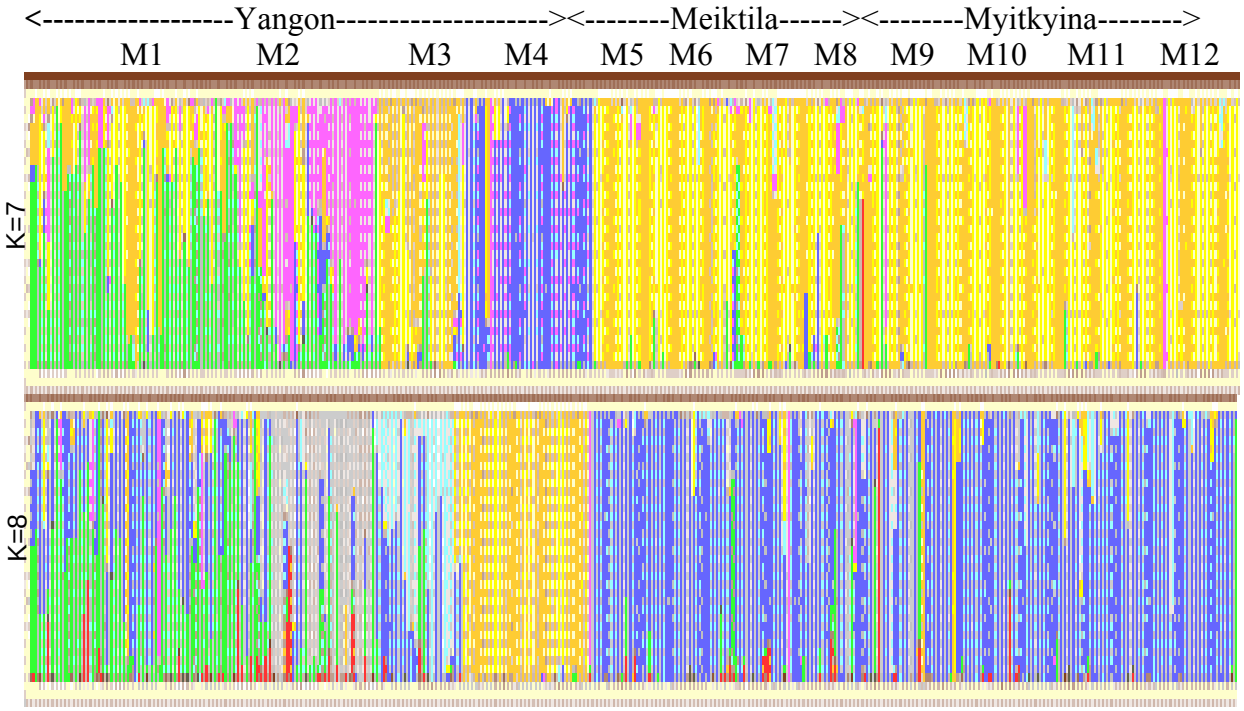


Fig. 2. Memberships of individuals to seven (upper panel) or eight (lower panel) genetic clusters estimated by TESS

between all populations. In TESS at k=8, the M4 population on the outskirts of Yangon became distinct. It was also found that the Yangon populations (M1-M3) were genetically heterogeneous compared to other populations (Fig. 2).

The results of the k- and g- tests for population bottlenecks are shown in Table 2. The number of loci that had positive values of k decreases when three clusters were pooled (five out of 13 loci) but none of the results were significant. All the g values were also all positive indicating that a model of constant population size could not be rejected for any of the population groupings.

Table 2. Population expansion analysis of *Aedes aegypti* microsatellite data in 13 loci

Sites	k-test (Number of positives)	g-test
Cluster 1 (Yangon)	7/13 NS	0.64
Cluster 2 (Meiktila)	7/13 NS	0.84
Cluster 3 (Myitkyina)	6/13 NS	0.65
Total (Myanmar)	5/13 NS	0.7

NS = not significant

DISCUSSION

The results show significantly low genetic structuring in *Aedes aegypti* at all spatial scales, which is consistent with the expectation that all these mosquitoes have a recent common ancestry in Africa [8]. The tests of population expansion indicate that there has not been a substantial bottleneck so that there have been multiple introductions from several different sources. However, the presence of genetic structuring within the groups may be the reason why the tests cannot detect a population bottleneck even if one has occurred.

Although the overall level of genetic differentiation is low, populations that are 5 km apart are significantly different from each other. The data indicate genetic structuring on a very small spatial scale (<500 m) in *Aedes aegypti*. The lack of signal of isolation by distance within a collection site indicates that individuals are not continuously distributed throughout the area (data not shown). Data from mark-release-recapture studies show that *Aedes*

aegypti mosquitoes have a limited flight range but can move up to a few hundred metres around their larval habitats [17].

Although such dispersal could be sufficient to genetically homogenise clusters within a 500m area, there are several factors which may prevent this. Dispersal rates may be reduced where there is increase in oviposition sites [25]. The genetic clustering of *Aedes aegypti* may therefore be due to clustering of oviposition sites and hosts around human habitation coupled with low dispersal.

Average F_{ST} values in this study were 0.026 and 0.032 at ~5 km and ~50 km scales (Table 1), respectively. Other microsatellite-based studies on a similar spatial scale had overall F_{ST} of 0.056 [26] and 0.053 [14] with many individual values being larger. One reason for low levels of genetic differentiation in this study compared to others could be due to the 500m sampling areas with multiple demes of highly clustered *Aedes aegypti* structure [27].

However, it is also possible that some other studies have overestimated as the larvae were sampled from areas as small as two to three [13] or four to five houses [26]. This could result in the over-representation of siblings in the sample. Overall, it is clear that *Aedes aegypti* has a highly clustered distribution and restricted gene flow on a very small spatial scale.

Although a signal of isolation by distance is detected, it is very slight (Fig. 1), indicating that the restricted mosquito dispersal at a small spatial scale does not explain larger scale population structure. This is in accord with results from the TESS analysis. It shows that although there is some regional clustering with similarities among populations, genetic clusters do not correspond obviously to spatial distance; some close populations are highly divergent (e.g. M4 population 50 km away from the main Yangon cluster). Conversely, populations that are distant may be very similar (e.g. Meiktila and Myitkyina clusters) (Fig. 2).

The genetic similarity of geographically distant populations, coupled with the greater genetic heterogeneity detected in the port city, Yangon, is indicative of some large scale dispersal events. The data provide good evidence that passive transportation of *Aedes aegypti* along human transportation routes is important in shaping large scale structure of this species. This has also been suggested based on previous population genetic studies in the southern United States [28] and SEA [13]. Passive dispersal likely involves the movement of immature stages of mosquito as well as adults; eggs, larvae and pupae could easily occur in water containers transported by people and the eggs can withstand desiccation for several months [16].

Our results have several implications for vector control. Conventional control measures such as insecticides or the removal of larval habitats are often implemented following a dengue outbreak [29]. The limited dispersal of *Aedes aegypti* indicates that this approach should be effective in removing infective populations and preventing their spread. It may also help to delay the spread of new insecticide resistance genes which is a major problem for vector control. However, the high selection pressure on such genes means that even a very small amount of dispersal enables the spread of insecticide resistance. To prevent this, effective monitoring of the emergence of insecticide resistance is essential. On the other hand, passive mosquito dispersal also plays an important role in shaping large scale genetic structure.

Conclusion

This study has shown low levels of genetic differentiation on all spatial scales in *Aedes aegypti*. It is still necessary to determine the exact spatial scale of genetic clusters and which environmental features may form the barriers between them. An effective approach will be landscape genetic studies in which individuals are sampled on a fine scale throughout

extensive urban and rural areas. It is also important to determine the environmental (including spatial) factors shaping large scale population structure and the extent to which these are historical or contemporary effects.

ACKNOWLEDGEMENT

We are grateful to our former Director-General and Deputy Director-General, DMR-LM, H.E. Deputy Minister, Prof. Paing Soe and Dr. Kyaw Min, and our present Director-General and Deputy Director-General, DMR-LM, Dr. Khin Pyone Kyi and Dr. Ye Htut for their kind support throughout this study. We acknowledge Prof. Terry Burke and Dr. Deborah Dawson from SMGF Laboratory, University of Sheffield for their kind advice and help. This study was funded by the WHO/TDR Collaborative Research Grant ID-A40198 and Research Training Grant (RTG) ID-A60987, and partly supported by NERC, UK.

REFERENCES

1. WHO. Mobilizing research to halt exponential growth of dengue. *TDR News* 2007; No. 77: 8-11.
2. Gubler DJ. Resurgent vector-borne diseases as a global health problem. *Emerging Infectious Diseases* 1998; 4: 442-450.
3. Gubler DJ. The global emergence/resurgence of arboviral diseases as public health problems. *Archives of Medical Research* 2002; 33: 330-342.
4. Kittayapong P. Malaria and dengue vector biology and control in Southeast Asia. In: *Bridging Laboratory and Field Research for Genetic Control of Disease Vectors.* B. G. J. Knols and C. Louis (eds). Springer Netherland. 2006; 111-127.
5. Prasittisuk C, Andjaparidze AG & Kumar V. Current status of dengue/dengue haemorrhagic fever in WHO Southeast Asia region. *WHO Dengue Bulletin* 1998; 22.
6. Thu HM, Lowry K, TT Myint, TN Shwe, AM Han *et al.*, Myanmar dengue outbreak associated with displacement of serotypes 2, 3, and 4 by dengue 1. *Emerging Infectious Diseases* 2004; 10: 593-597.
7. Smith CEG. The history of dengue in tropical Asia and its probable relationship to the mosquito *Aedes aegypti*. *Journal of Tropical Medicine and Hygiene* 1956; 59: 243-251.
8. Tabachnick WJ, & Powell JR. Worldwide survey of genetic variation in the yellow fever mosquito, *Aedes aegypti*. *Genetical Research* 1979; 34: 215-229.
9. WHO. Dengue and dengue haemorrhagic fever fact sheet No. 117 (Revised in May 2008) Available from URL: http:// www.who.int/ mediacentre/factsheet
10. Balloux F & Lugon-Moulin N. The estimation of population differentiation with microsatellite markers. *Molecular Ecology* 2002; 11: 155-165.
11. Ravel S, Monteny N, Olmos DV, Verdugo JE & Cuny G. A preliminary study of the population genetics of *Aedes aegypti* (Diptera: Culicidae) from Mexico using microsatellite and AFLP markers. *Acta Tropica* 2001; 78: 241-250.
12. Ravel S, Herve JP, Diarrassouba S, Kone A & Cuny G. Microsatellite markers for population genetic studies in *Aedes aegypti* (Diptera: Culicidae) from Cote d'Ivoire: evidence for a microgeographic genetic differentiation of mosquitoes from Bouake. *Acta Tropica* 2002; 82**:** 39-49.
13. Huber K, Le Loan L, Chantha N &. Failloux A B. Human transportation influences *Aedes aegypti* gene flow in Southeast Asia. *Acta Tropica* 2004; 90: 23-29.
14. Paupy C, Chantha N, Huber K, Lecoz N, Reynes JM *et al.*, Influence of breeding sites features on genetic differentiation of *Aedes aegypti* populations analyzed on a local scale in Phnom Penh Municipality of Cambodia. *American Journal of Tropical Medicine and Hygiene* 2004; 71: 73-81.
15. Manel S, Schwartz MK, Luikart G & Taberlet P. Landscape genetics: combining landscape ecology and population genetics. *Trends in Ecology and Evolution* 2003; 18: 189-197.
16. Christophers SR. *Aedes aegypti (L.), the yellow fever mosquito*. Cambridge University Press, London. 1960.
17. Reiter P, Amador MA, Anderson RA & Clark GG. Dispersal of *Aedes aegypti* in an urban area after blood feeding as demonstrated by rubidium- marked eggs. *American Journal of Tropical Medicine and Hygiene* 1995; 52: 177-179.
18. Sambook J & Russell DW. *Molecular cloning: a laboratory manual*. Cold Spring Harbor Laboratory Press, New York, 2001.
19. Slotman MA, Kelly NB, Harrington LC, Kitthawee S, Jones JW *et al.* Polymorphic microsatellite markers for studies of *Aedes aegypti* (Diptera: Culicidae), the vector of

dengue and yellow fever. *Molecular Ecology Notes* 2007; 7**:** 168-171.

20. Excoffier L, Laval G & Schneider F. *An integrated software package for population genetics data analysis. User manual*. Computational and Molecular Population Genetics Lab (CMPG), Institute of Zoology, University of Berne, Berne, 2006.
21. Slatkin M. Gene flow and the geographic structure of natural populations. *Science* 1987; 236**:** 787-792.
22. Francois O, Ancelet S & Guillot G. Bayesian clustering using hidden Markov random fields in spatial population genetics. *Genetics* 2006; 174: 805-816.
23. Reich DE, Feldman MW & Goldstein DB. Statistical properties of two tests that use multilocus data sets to detect population expansions. *Molecular Biology Evolution* 1999; 16: 453-466.
24. Bilgin R. Kgtests: a simple Excel Macro program to detect signatures of population expansion using microsatellites. *Molecular Ecology Notes* 2007; 7**:** 416-417.
25. Edman JD, Scott TW, Costero A, Morrison AC, Harrington LC *et al. Aedes aegypti* (Diptera: Culicidae) movement influenced by availability of oviposition sites. *Journal of Medical Entomology* 1998; 35: 578-583.
26. Huber K, Le Loan L, Hoang TH, Ravel S, Rodhain F *et al.* Genetic differentiation of the dengue vector, *Aedes aegypti* (Ho Chi Minh City, Vietnam) using microsatellite markers. *Molecular Ecology* 2002; 11: 1629-1635.
27. Wright S. Systems of mating: the effects of inbreeding on the genetic composition of a population. *Genetics* 1921; 6: 124-143.
28. Merrill SA, Ramberg FB & Hagedorn HH. Phylogeography and population structure of *Aedes aegypti* in Arizona. *American Journal of Tropical Medicine and Hygiene* 2005; 72: 304-310.
29. WHO. Situation of dengue/DHF in the Southeast Asia region: Prevention and control status in SEA countries. 2006 [www.searo.who.int/en/Section10/Section332_1099.htm]
30. James AA, Benedict MQ, Christophides GK, Jacobs-Lorena M & Olson KE. Evaluation of drive mechanisms (including transgenes and drivers) in different environmental conditions and genetic backgrounds. In: *Bridging Laboratory and Field Research for Genetic Control of Disease Vectors.* B. G. J. Knols and C. Louis (eds). Springer Netherland, 2006; 149-155.
31. Thomas DD, Donnelly CA, Wood RJ &. Alphey LS. Insect population control using a dominant, repressible, lethal genetic system. *Science* 2000; 287: 2474-2476.

The Myanmar Health Sciences Research Journal, Vol. 22, No. 1, 2010

Cloning of the hepatitis B surface antigen gene (3): integration of the recombinant HBsAg gene-containing plasmid into genomic DNA of *Pichia pastoris* yeast cells

**Win Aung, **Yeon-Hee Kim, **Hyunah Kim, **Suk-Hoon Ha & *Khin Pyone Kyi*

*Diagnostics and Vaccine Research Centre
Department of Medical Research (Lower Myanmar)
*Institute of Science and Technology
CJ Corporation, Ichon, Republic of Korea

The majority of currently available recombinant hepatitis B vaccines in the market have been produced by using appropriate expression plasmids in *Saccharomyces cerevisiae* and *Hansenula polymorpha* yeast cells as host systems. Recently, *Pichia pastoris*, one of the methylotrophic yeast cells has been used as an alternative host for cloning of yeast expression plasmid carrying the hepatitis B surface antigen (HBsAg) structural gene and as a more productive host for production of recombinant hepatitis B vaccines. In previous studies, purification and transformation of the HBsAg gene into the competent *E. coli* cell, and amplification and ligation of HBsAg gene with yeast plasmid followed by transformation into the competent *E. coli* cells were successfully carried out. In this study, attempts were made to extract the above closed-circular yeast plasmid containing recombinant HBsAg gene from transformant *E. coli* cell and to linearize by using restriction enzymes, *Sac* I and *Sal* I, followed by transformation and integration of it into the competent final host, *Pichia pastoris* yeast cells, for further expression of the HBsAg protein. The results of the study were confirmed by direct PCR identification, restriction enzyme analysis followed by agarose gel electrophoresis determination and DNA sequencing analysis. It is expected that *Pichia pastoris* yeast cell could be used as a promising host system for production of recombinant hepatitis B vaccines in the near future.

INTRODUCTION

Recombinant DNA technology has revolutionized biology and is having an ever increasing impact on clinical medicine and pharmaceutical industry. The primary objective of gene cloning for biotechnological applications is the expression of the cloned gene in a selected host organism. Prokaryotic expression systems are generally useful for producing heterogenous (recombinant) proteins from cloned eukaryotic cDNAs. In some cases, bacteria are either unstable or lack biological activity. In addition, despite careful purification procedures, bacterial compounds that are toxic or that cause a rise in body temperature in humans and animals (pyrogens) may contaminate the final product. To avoid these problems, eukaryotic expression systems for the production of proteins were investigated and successfully developed using yeast species [1].

For development of currently available recombinant hepatitis B (HB) vaccines, first, HB virus recovered from plasma of a hepatitis B carrier has been used to prepare viral DNA: that DNA has been cloned in *E. coli* and the gene coding for HBsAg has been isolated. This gene has been inserted into yeasts by means of appropriate expression vectors. Purified HBsAg proteins expressed from transfected yeast cells containing it have been formulated into HB vaccines and shown to induce protective antibody response in human [2].

Since 1986 to date, *Sacchromyces cerevisiae* and *Hansenula polymorpha* have been used as host systems for commercial production of the majority of recombinant HB vaccines. Recently, an increasing number of publications attest to the favorable properties of methylotrophic yeasts as hosts for heterologous gene expression. Among them, *Pichia pastoris* is emerging as a heterologous protein expression system and the most significant industrial application for production of heterologous recombinant proteins. In many instances, the heterologous proteins were produced at much higher levels in *P. pastoris* when compared with the respective productivity in traditional *S. cerevisiae* and *H. polymorpha* yeast cells [3, 4, 5, 6].

In our recent two preliminary studies, we first extracted the genomic DNA of *H. polymorpha* transformant cell from which the HBsAg gene was amplified by using PCR followed by ligation with pGEM-T vector and transformation into the competent *E. coli* DH5 alpha cell [7]. Then, the HBsAg gene was again amplified, digested with specific restriction enzymes (RE) and ligated with pPIC9K vector which has been successfully transformed into the competent *E. coli* DH5 alpha cell [8].

In this study, attempts were made to linearize the above closed-circular plasmid containing recombinant HBsAg gene by using specific restriction enzymes, and to transform it into the competent final host, *Pichia pastoris* yeast cells for further expression of the HBsAg protein with an aim to produce recombinant HB vaccine locally in the near future.

MATERIALS AND METHODS

HBsAg-containing plasmid

The purified, closed-circular plasmid (~10,000 base pairs) containing the recombinant HBsAg gene (678 bp) and pPIC9K vector (9276 bp) in the competent *E. coli* DH5 alpha cells was locally prepared in the laboratory of Research and Development Centre of Pharmaceuticals, Institute of Science and Technology, CJ Corporation, Ichon City, Republic of Korea, prior to the transformation procedure [7&8].

Host strains

Commercially available lyophilized form of *P. pastoris* GS 115 (his 4) strains derived from the wild type NRRL Y-11430 of the In vitrogen, Life Technologies was used in cloning technique for plasmid amplification (Fig.1).

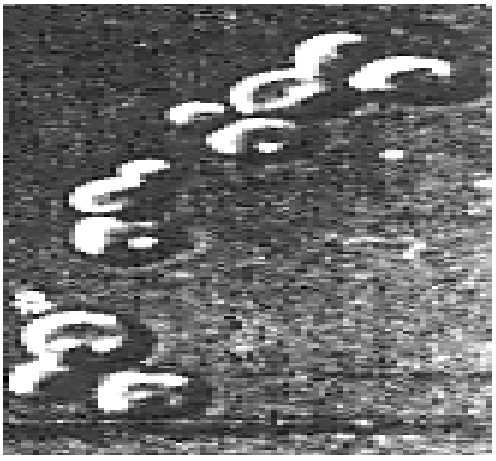


Fig. 1. Morphology of the *P. pastoris* yeast cells

Preparation of the competent P. pastoris yeast cell (spheroplast)

The competent *P. pastoris* yeast cell was prepared in our laboratory from wild type *P. pastoris* cell GS 115 by spheroplasting procedure using 5% zymolyase and 1M sorbitol, described in the manual of Multi-Copy *Pichia* Expression Kit [9]. This procedure provides the highest efficiency of transformation in which the cell wall of *P. pastoris* is partially digested by using zymolyase, a β-glucanase that hydrolyses the glucose polymers with β 1-3 linkages in the cell wall. After transformation, these cells are suspended in sorbitol to facilitate cell wall regeneration.

Transformation and integration of the recombinant HBsAg gene into P. pastoris cells

The purified, closed-circular recombinant DNA was subjected to RE digestion with *Sac* I and *Sal* I separately. *Sac* I was used for insertion at AOX1 and *Sal* I was used

for insertion at *HIS 4*. These restriction sites are not present in the HBsAg gene but present in pPIC9K vector. The linearized DNA construct was extracted by using phenol: chloroform: isoamyl alcohol method [10], and confirmed by 1.5% agarose gel electrophoresis and quantified by spectrophotometric determination. The purified linearized recombinant DNA was further transformed into the competent *P. pastoris* yeast cell, i.e spheroplast [9].

The efficient transformation of *P. pastoris* was identified by an appearance of transformant cell apparently grown in the regeneration Dextrose Broth without histidine agar plate 4-6 days after cultivation at 30^{o}C. The transformation was also confirmed by direct colony PCR (using specific primers: 5' AOX 1 (GAC TGG TTC CAA TTG ACA AGC) and 3' AOX1 (GCA AAT GGC ATT CTG ACA TCC), followed by agarose gel electrophoresis [11].

For PCR analysis, restriction sites of primers for 5' AOX1 and 3'AOX1 are 855-897 bp and 1327-1347 bp, respectively, in pPIC9K vector. The genomic DNA of *P. pastoris* transformant was also extracted by using the QAIGEN test kit [12]. Then the extracted DNA of interest was amplified by using specific primers; AY21F (5'-ATG GAG AAC ATC ACA TCA GGA-3') and CTR (5'-TGG GAT TGT TTT GTT TCT C-3'), followed by PCR purification, agarose gel electrophoresis determination and DNA sequencing analysis [13]. The restriction sites of primers for AY21F and CTR are 1-21 bp in the HBsAg gene and 679-697 bp in the extra flanking sequence, respectively. Since CTR primer is used for amplification of the extra-sequence, of the HBsAg gene, the size of nucleotide sequence of amplified fragment is larger than 678 bp.

Screening of multi-copy insert in Pichia transformants

It was performed by culturing of *P. pastoris* transformants on the Yeast Peptone Dextrose (YPD) plates with different concentrations of geneticin ranging from 0, 0.25, 0.5, 0.75, 1, 1.5, 1.75, 2, 3 and 4 mg/ml. These plates were incubated at 30^{o}C and checked for viable colonies after5days. The geneticin hyper-resistant colonies were observed as multi-copy inserts [9].

RESULTS

Electrophoretic identification of the recombinant plasmid (pPIC9K+HBsAg gene) before and after restriction enzyme digestion with *Sac* I and *Sal* I is shown in Fig. 2.

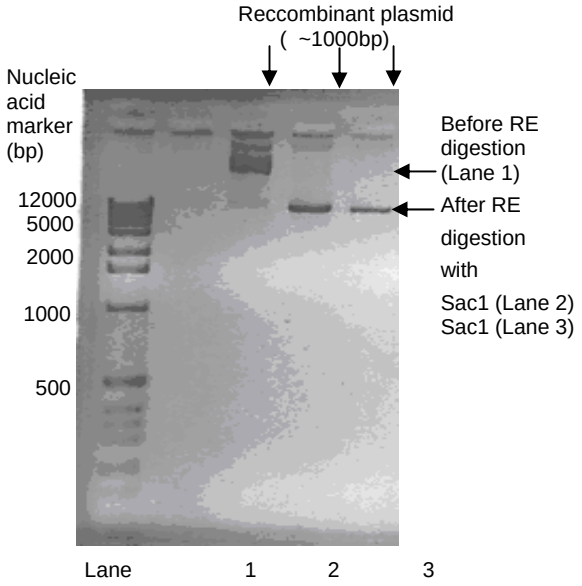


Fig. 2. Electrophoretic identification of the recombinant plasmid before and after RE digestion with *Sac* I and *Sal* I

The linearized recombinant plasmids were observed as slightly faster mobility than that of the closed-circular recombinant plasmid. Fig. 3 demonstrates the direct PCR identification of the HBsAg DNA fragment (~1.2 K bp) which contains ~700 bp of HBsAg segment and ~490 bp of flanking region, in the *P. pastoris* integrant on agarose gel electrophoresis determination. It indicated that recombinant DNA containing the HBsAg gene was successfully transformed into the competent *P. pastoris* yeast cell.

Fig. 4 shows the agarose gel electrophoretic identification of the HBsAg DNA segment, amplified from chromosomal DNA of transformant *P. pastoris* cell. The nucleotide

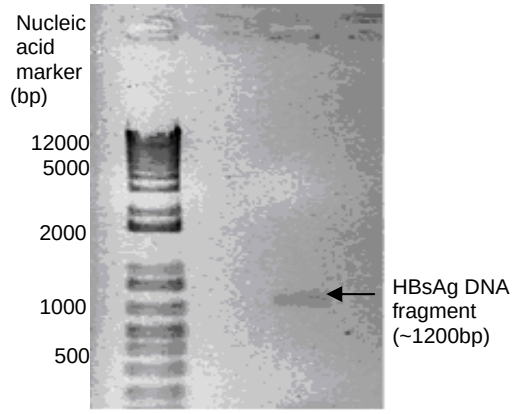


Fig. 3. Direct PCR identification of the HBsAg gene in *P. pastoris* transformant using 5'AOX 1 and 3'AOX 1 primers

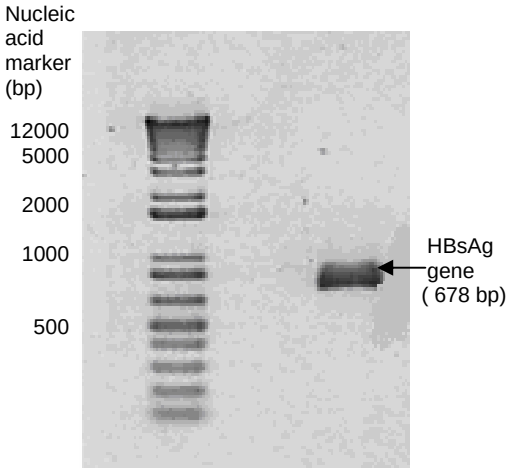


Fig. 4. Electrophoretic identification of the HBsAg gene, amplified from genomic DNA of *P. pastoris* transformant using AY21F and CTR primers

sequence of the purified HBsAg DNA segment, amplified from chromosomal DNA of transformant *P. pastoris* cell, analyzed by using the ABI Prism 3100 Genetic Analyzer is illustrated in Fig. 5. The nucleotide sequences (678 bp of HBsAg & 19 bp of extra-sequence) obtained were found to be totally identical to that of the HBsAg gene initially amplified from chromosomal DNA of *H. polymorpha* cell [7]. Table 1 depicts that the colonies containing multi-copy HBsAg gene inserts in *P. pastoris* transformants were observed only up to the YPD plate with geneticin concentration of 0.75 mg/ml from which these colonies can be used for further process of HBsAg protein expression.

(AY2IF)

ATGGAGAACA TCACATCAGG ATTCCTAGGA CCCCTGCTCG TGTTACAGGC
GGGGTTTTTC TTGTTGACCA GAATCCTCAC AATACCGCAG AGTCTAGACT
CGTGGTGGAC TTCTCTCAAT TTTCTAGGGG GAACTACCGT GTGTCTTGGC
CAAAATTCGC AGTCCCCAAC CTCCAATCAC TCACCAACCT CCTGTCCTCC
AACTTGTCCT GGTTATCGCT GGATGTGTCT GCGGCGTTTT ATCATCTTCC
TCTTCATCCT GCTGCTATGC CTCATCTTCT TGTTGGTTCT TCTGGACTAT
CAAGGTATGT THCCCGTTTTG TCCTCTAATT CCAGGATCTT CAACTACCAG
CACGGGACCA TGCAGAACCT GCAGACTCC TGCTCAAGGA ACCTCTATGT
ATCCCTCCTG TTGCTGTACC AAACCTTCGG ACGGAAATTG CACCTGTATT
CCCATCCCAT CATCCTGGGC TTTCGGAAAA TTCCTATGGG AGTGGGCCTC
AGCCCGTTTC TCCTGGCTCA GTTACTAGT GCCATTTGTT CAGTGGTTCG
TAGGGCTTTC CCCCACTGTT TGGCTTTCAG TTATATGGAT GATGTGGTAT
TGGGGGCCAA GTCTGTACAG CATCTTGAGT CCCTTTTTAC CGCTGTTACC
AATTTTCTTC TGTCTTTGGG TATACATT GA GAAACAAAAC AATCCCA

Extra sequence (CTR)

Fig. 5. Nucleotide sequence of the HBsAg gene extracted from *P. pastoris* transformant using AY21F and CTR primers

Table 1. Screening of multi-copy inserts in Pichia transformants

Concentrations of geneticin in YPD plates (mg/ml)	Growth of pichia transformant colonies observed after 5 days
0.25	Present
0.5	Present
0.75	Present
1.0	Absent
1.5	Absent
1.75	Absent
2.0	Absent
3.0	Absent
4.0	Absent

DISCUSSION

Yeasts are eukaryotic, unicellular, microscopic and usually spherical in shape with diameter of 3-15 µm. *Pichia pastoris* is one of the methylotrophic yeast cells, capable of metabolizing methanol as its sole energy and carbon source. They appear as white or cream colonies with 1-3mm in size, multilateral budding without filament containing 1-4 hat-shaped ascospores. The optimal growth temperature of Pichia is 28^{o}C -30^{o}C and high pH affects its growth [14].

The heterologous expression in *Pichia* can be either intracellular or secreted. The major advantage of expressing heterologous protein as secreted protein is that it secretes

very low level of native proteins. Therefore, the secreted heterologous protein comprises the vast majority of the total protein in the medium and serves as the first step in purification of protein [3]. *P. pastoris* GS115 host strain has a mutation in the histidinol dehydrogenase gene (*his4*) that prevents them from synthesizing histidine.

P. pastoris cells are histidine deficient (i.e. His) and well grow only on complex medium YPD and minimum medium such as Yeast Nitrogen Base (YNB) supplemented with histidine. Until transformed, *P. pastoris* GS 115 cannot grow on histidine deficient medium. They can grow on the histidine deficient medium only after transformation with plasmid DNA pPIC9K which has the *HIS 4* gene that complements *his 4* gene in the host. So, transformants are selected for their ability to grow on the histidine deficient medium. *HIS 4* provides a selectable marker to isolate *Pichia* recombinant strain. The pPIC9K is super-coiled plasmid vector, utilizing the strong methanol inducible AOX1 promoter for expression of heterologous protein and secretes recombinant into the medium. It also contains the bacterial kanamycin (Kan) gene that confers resistance to geneticin in *Pichia*. A single copy of pPIC9K integrated into the *Pichia* genome confers resistance to geneticin to a level of 0.25 mg/ml. The level of geneticin resistance roughly depends on the number of Kan resistance gene integrated.

Thus, the presence of the *Kan* gene can be used as a tool to detect transformants that harbour multicopies of the gene. One can infer from geneticin hyper-resistance that the clone contains multi-copy of the gene-inserted [9]. Recently, an increasing number of publications attest to the favorable properties of methylotrophic yeasts as hosts for heterologous gene expression. Among them, *P. pastoris* is emerging as the most significant industrial application for production of heterologous recombinant proteins.

In our previous studies, an initial plasmid construction was usually performed in *E. coli* cells before transformed into *P. pastoris* yeast cells since the expression plasmid pPIC9K has been transformed into *E. coli* cells to have a permanent stock of plasmid DNA and this plasmid construction can be maintained in *E. coli* strain [7&8]. In this study, the closed-circular recombinant HBsAg gene was first linearized prior to the transformation into *P. pastoris* yeast cell. The linearization with specific restriction enzymes procedure is essential since linear DNA can generate a stable transformant between DNA of interest and regions of homology within the genome. Such integrants show extreme stability in the absence of selective pressure even when present as multiple copies. Although the transformation without RE digestion can be done, this event of non-linearized plasmid trans-formation is at a lower frequency [4].

In our study, linearization with *Sac* I will generate recombination at *AOX1* locus and that with *Sal* I will generate recombination at *HIS 4* locus. Besides, single cross over events (insertion) are much more likely to happen than double cross over events (replacement). Multiple insertion events occur spontaneously at about 1-10% of the single insertion events. In our study, colonies containing maximum multi-copy HBsAg gene inserts in *P. pastoris* transformants were generally observed at the YPD plate with geneticin concentration of 0.75 mg/ml (Table 1). However, the concentration levels of geneticin resistance may not necessarily correlate with the number of gene copy-insert. Therefore, actual number of gene copies in Pichia recombinant needs to be determined by use of quantitative dot blot analysis, Southern blot analysis and differential hybridization [15].

In this study, the HBsAg gene-containing plasmid pPIC9K was successfully transformed into the competent *Pichia* yeast cell, usually integrated into its *genome*. The results from each and every steps were confirmed by direct PCR identification, RE analysis followed by agarose gel electrophoresis determination and DNA sequencing

analysis. Finally, the geneticin hyper-resistance clones of *Pichia* transformant, containing multi-copies of the HBsAg gene were selected for further expression of the HBsAg protein. This study described the use of *P. pastoris* as a possible host for cloning of the HBsAg gene-containing plasmid and also for heterologous expression of the HBsAg protein.

In conclusion, we have successfully developed a suitable and alternative host for the biological synthesis of HBsAg protein in the *P. pastoris* as a production organism for recombinant pharmaceuticals. Therefore, the recombinant HB vaccines derived from *P. pastoris* host are expected to enter the drug market, thus competing in selective field with the traditional *S. cerevisiae* and *H. polymorpha* yeast cells. In the near future, *P. pastoris* will become a popular host organism for the expression of HBsAg protein to levels of industrial value.

REFERENCES

1. Gliok BR & Pasternak JJ. Heterogenous protein production in eukaryotic cells. In: *Molecular Biotechnology: Recombinant DNA technology* 1994; 13-131.
2. WHO. Requirements for hepatitis B vaccines made by recombinant DNA technology. *WHO Technical Report Series* 1989; 786:38-60.
3. Barr KA, Hopkins SA & Sreekrishna K. Protocol for Efficient Secretion of HSA developed from *Pichia pastoris*. *Pharm. Eng* 1992; 12: 48-51.
4. Cregg JM, Barringer KJ & Hessler AY. *Pichia pastoris* as a host system for transformation *Molecular and Cell Biology* 1985; 5:3376-3385.
5. Cregg JM & Huggins DR. Production of foreign proteins in the yeast *Pichia pastoris. Canadian Journal of Botany Supplement* 1995; 73:5981-5987.
6. Creeg JM, Vedvick TS & Raschke WC Recent advances in the expression of Foreign Genes in *Pichia pastoris. Biotechnology* 1993; 11: 905-910.
7. Win Aung, Yeon-Hee Kim, Hyunah Kim, Suk-Hoon Ha & Khin Pyone Kyi Cloning of the hepatitis B surface antigen gene (1) extraction and transformation of the recombinant HBsAg gene containing plasmid into the *E. coli* DH 5 alpha cells. *Myanmar Health Sciences Research Journal* 2009; 21 (1):56-61.
8. Win Aung, Yeon-Hee Kim, Hyunah Kim, Suk-Hoon Ha & Khin Pyone Kyi. Cloning of the hepatitis B surface antigen gene (2): amplification, restriction enzyme digestion and ligation of HBsAg gene and yeast plasmid pPIC9K. *Myanmar Health Sciences Research Journal 2009*; 2 (3): 126-131.
9. Manual of the Multi-copies Pichia Expression Kit: For the isolation and expression of recombinant proteins from *Pichia pastoris* containing multi-copies of a particular gene, 2001.
10. Sambrook & Russell. Protocol 5. Preparation of genomic DNA from mouse tail and other small samples. In: *Molecular Cloning: A Laboratory Manual* 2001; 1: Third edition, 6.23- 6.27.
11. Linder S, Schliwa M & Kube-Granderath E. Direct PCR screening of *Pichia pastoris* Clones. *Bio Technique* 1996; 20: 980-982.
12. Handbook of the QIAGEN Genomic DNA 1995.
13. Sequencing Chemistry Guide for ABI Prism 3100 Genetic Analyzer, HITACHI, 1-21.
14. Barnett JA, Payne RW & Yarrow D. Yeasts: characteristics and identification. Published by the Press Syndicate of the University of Cambridge USA, 1983.
15. Brierley RA, Davis GR & Holtz GC. Production of insulin-like growth Factor -1 in Methylotropic yeast cells. United States Patent 5324639, 994.

The Myanmar Health Sciences Research Journal, Vol. 22, No. 1, 2010

Erythrocyte antioxidant enzymes activities and plasma carbonyl protein levels in smokers

**Khin Than Yee, **Kyu Kyu Maung, **Myo Win,*
****Theingi Myint, *Kyaw Oo & *Zaw Myint*

*Biochemistry Research Division
Department of Medical Research (Lower Myanmar)
**University of Medicine (1), Yangon
***University of Medicine, Mandalay

Smoking has been implicated in the pathogenesis of ischemic heart disease, chronic obstructive lung diseases and cancers. More than 4700 chemical compounds of cigarette smoke, including free radicals, oxidants and reducing agents have been identified. The aim of this study is to investigate the effect of cigarette smoking on the levels of erythrocyte antioxidant enzymes and plasma carbonyl protein which can be used as a marker of protein oxidation. Thirty-two smokers and 36 apparently healthy control subjects from staff of University of Medicine (1) and Department of Medical Research (Lower Myanmar) and blood donors of National Blood Centre, Yangon were studied. The subjects were males aged between 25-40 years. Erythrocyte superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities and plasma carbonyl protein levels were measured using a spectrophotometer. The erythrocyte CAT activity was significantly lower in cigarette smokers (264.57±108.37 U/g Hb) compared with non-smokers (473.59±267.87 U/g Hb) ($p<0.005$). There were no significant differences in erythrocyte SOD and GPx activities and plasma carbonyl protein levels between the two groups. There was a positive correlation ($r=+0.35$, $p<0.05$) between the number of cigarettes consumed per day and CAT activity in smokers. In conclusion, erythrocyte CAT activity was reduced in cigarette smokers due to the free radicals present in cigarette smoke.

INTRODUCTION

Cigarette smoking is the largest growing cause of death in the world [1]. Tobacco smoking is a risk factor for 6 of 8 leading causes of death including ischaemic heart diseases, cardiovascular diseases, lower respiratory infection, chronic obstructive pulmonary diseases, tuberculosis and trachea, bronchus and lung cancer in the world [2]. It has been estimated that a single puff of a cigarette contains as much as 10^{15} gas phase radicals and 10^{14} tar phase radicals potentially capable of modifying macromolecules including lipids, proteins and DNA [3].

The defense systems within the body limit the levels of reactive oxidants and the damage they inflict. The three main enzymes that control the biological effects of reactive oxygen species are superoxide dismutase (SOD), which catalyses dismutation of the superoxide anion into H_2O_2, catalase (CAT), which detoxifies H_2O_2, and glutathione peroxidase (GPx), which reduces organic peroxides to their alcohols [4]. However, when free radicals are generated in excess or the cellular antioxidant defense system is defective, they can stimulate chain reactions by interacting with proteins, lipids and nucleic acids, causing cellular dysfunction and even death [5].

Proteins are major targets for reactive oxidants in cells and oxidized proteins accumulate during aging, oxidative stress and in some pathological conditions. Exposure of proteins to reactive oxygen species results in modification of amino acid residues, which alters protein structure and function [6]. It is reported that in vitro exposure of plasma to gas-phase cigarette smoke leads to the rapid accumulation of plasma protein carbonyls [7].

The circumstantial data from in vitro studies and measurements of antioxidant levels in smokers suggested that cigarette smoke may cause oxidative injury. From the literature, the findings are in disagreement over the erythrocyte SOD, GPx and CAT activity in smokers. The present study was conducted firstly to find out the effect of cigarette smoking on erythrocyte antioxidant enzymes activities and a selected marker of oxidative damage, and secondly, to verify the possible correlation between the smoking status and the erythrocyte anti-oxidant enzymes activities due to cigarette smoking.

MATERIALS AND METHODS

A comparative, cross-sectional study was carried out on a total of 68 male subjects aged between 25-40 years (32 smokers and 36 non-smokers). Smokers were defined as those who, at the time of survey, smoked any tobacco product at least once a day daily for at least 3 year duration while non-smokers were those who do not smoke at all [8]. All subjects were apparently healthy volunteers recruited from the staff of University of Medicine (1), Department of Medical Research (LM) and blood donors at National Blood Centre, Yangon.

Informed consents were obtained from all subjects. The subjects were firstly explained the nature and procedure of the study. They were not suffering from any diseases, were not on any medication, including vitamins and were not taking excess alcohol. These facts and smoking status of the subjects were asked with questionnaires.

Venous blood was collected in tubes with EDTA and centrifuged at 3000 rpm, 30°C for 10 minutes. Erythrocytes were washed 3 times with 3 ml of cold isotonic saline solution. Then, the plasma and the packed cells were stored at -80°C until analysis. The plasma was used for the determination of carbonyl protein. The packed cells were lysed in 4 times of its volume of deionized water by vortex-mixing. Membrane-free haemolysate was obtained by centrifugation at 3000 rpm, 4°C for 10 minutes. The clear supernatant was collected as haemolysate to determine SOD, GPx, CAT activities and hemoglobin concentration.

Hemoglobin concentration was determined by cyanmethaemoglobin method [9]. Erythrocyte catalase activity was determined with the method of Aebi [10]. The principle of the CAT assay was based on the determination of the rate constant (s^{-1}, k) of H_2O_2 decomposition rate. Rate constant, $k=(1/\Delta t)xIn\ (A_1/A_2)$, where A_1 and A_2 were the absorbance values of H_2O_2 at times t_1 and t_2. The initial rate of disappearance of H_2O_2 (0-40 seconds) was recorded spectrophotometrically at a wavelength of 240 nm. One unit of catalase activity was defined as the rate constant of the first-order reaction. The catalase activity was expressed as unit per gram hemoglobin (U/g Hb).

Erythrocyte glutathione peroxidase activity was determined by using the method of Takahashi [11]. The GPx activity was determined spectrophotometrically in erythrocyte haemolysates using an indirect coupled assay. The GPx catalyzes the oxidation of glutathione by t-butyl hydroperoxide. In the presence of glutathione reductase and nicotiamide adenine dinucleotide phosphate (NADPH), the oxidized glutathione is immediately converted to the reduced form with a concomitant oxidation of NADPH to $NADP^+$. The change in absorbance at 340 nm was measured. One unit of activity was defined as the number of NADPH oxidized per minute, using a molar coefficient of extinction of $6.22x10^6\ mM^{-1}cm^{-1}$ for

NADPH. Erythrocyte GPx activity was expressed as U/g Hb.

Erythrocyte superoxide dismutase activity was determined according to the procedure of Suzuki [12]. The principle of SOD activity measurement was based on the inhibition of nitroblue tetrazolium reduction with the xanthine-xanthine oxidase system as a superoxide generator. Superoxide radicals produced reduce nitroblue tetrazolium and form formazan. SOD prevents this reaction, and its activity is inversely proportional to the absorbance value of formazan at 560 nm. One SOD unit was defined as the amount of the enzyme causing 50% inhibition of the nitroblue tetrazolium reduction rate. SOD activity was expressed in U/g Hb.

Plasma carbonyl protein was determined as described by Reznick and Packer [13]. Carbonyls were determined by using dinitrophenylhydrazine (DNPH) dissolved in HCl, accompanied by blank in HCl alone. After the DNPH reaction, proteins were precipitated with an equal volume of 20% (w/v) trichloroacetic acid and the pellets were washed once with 2 ml of 10% (w/v) trichloroacetic acid and 3 times with 2 ml of an ethanol/ethyl acetate mixture (1:1).

Washings were achieved by mechanical disruption of the pellets in washing solution and re-pelleting by centrifugation at 3000 rpm for 5 minutes. Finally, the precipitates were dissolved in 6M guanidine-HCl solution and the absorbance at 370 nm was determined. Protein contents were determined on the HCl blank pellets using a bovine serum albumin standard curve in guanidine-HCl and reading the absorbance at 280 nm.

All results are presented as mean±SD. Differences in means between groups were analyzed by un-paired 't' test. Correlation statistics between variables were assessed by calculating the Pearson coefficient. Differences were considered statistically significant at $p<0.05$.

RESULTS

Table 1 shows the smoking status of the studied population. They smoked 3-15 cigarettes per day (9.09±3.76 cigarettes/ day) and the time duration they smoked was 3-22 years (9.87 ± 5.62 years).

Table 1. Smoking status of the studied population (n=32)

	Range	Mean±SD
Age (years)	25-40	31.77 ± 4.67
No. of cigarettes/day	3 -15	9.09 ± 3.76
Duration of smoking (years)	3 -22	9.87 ± 5.62

Table 2 shows the erythrocyte antioxidant enzymes activities and plasma carbonyl protein levels in smokers and non-smokers. Erythrocyte catalase activity was significantly decreased ($p<0.001$) in smokers when compared with non-smokers. It was found that smokers had a lower erythrocyte SOD activity than non-smokers but this difference did not reach the level of significance. The mean erythrocyte gluthathione peroxidase level was higher in smokers than that in non-smokers but they were not significantly different. The mean plasma carbonyl protein levels in smokers and non-smokers were not also different.

Table 2. Erythrocyte antioxidant enzymes activities and plasma carbonyl protein levels in smokers and non-smokers

	Smokers (n = 32)	Non-smokers (n = 36)
Age (years)	31.77±4.67	31.96±5.50
Superoxide dismutase (U/gHb)	679.87±324.28	757.83±373.33
Catalase (U/gHb)	264.57±108.37*	473.59 ±267.87
Glutathione peroxidase (U/gHb)	76.65±27.86	68.74±23.02
Carbonyl protein (nmol/mg protein)	1.41±0.83	1.41±0.70

*$p<0.001$

The results of correlation analysis between the activities of the studied enzymes and the number of cigarettes consumed per day and smoking duration are given in Table 3.

Table 3. Correlation between smoking status and erythrocyte superoxide dismutase and glutathione peroxidase activities in smokers

Smoking status	Correlation coefficients of erythrocyte antioxidant enzymes	
	SOD	GPx
No. of cigarette per day (3-15 cigarettes/day)	-0.019	-0.141
Duration of smoking (3-22 years)	0.04	-0.155

Both the superoxide dismutase and glutathione peroxidase activities have negative correlation with number of cigarette smoked per day. The superoxide dismutase has positive correlation with the duration of smoking but glutathione peroxidase has negative correlation with the duration of smoking.

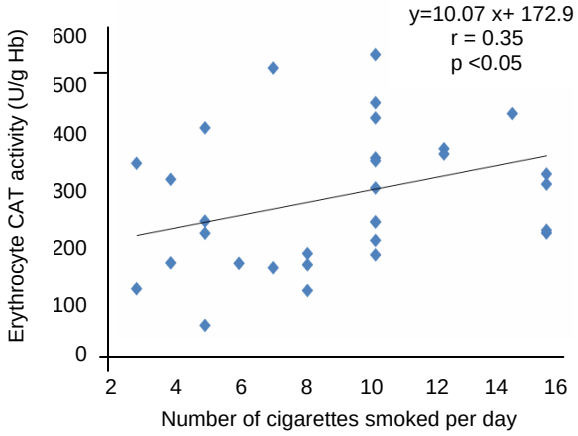


Fig. 1. Correlation between numbers of cigarettes smoked per day and erythrocyte catalase activity among smokers

There was significant positive correlation between the number of cigarettes smoked per day and erythrocyte CAT activity ($r=0.35$, $p<0.05$) in smokers. Figure 1 shows the moderate association between number of cigarettes smoked per day and erythrocyte CAT activity in smokers.

DISCUSSION

Cigarette smoke contains many oxidants, free radicals and carcinogenic substances that are capable of reacting with, or inactivating, the essential cellular constituents. These potentially toxic substances are inactivated or scavenged by the antioxidants before they can inflict damage to lipids, proteins or nucleic acids. Superoxide dismutase, catalase and glutathione peroxidase are generally believed to play a vital role in protecting the body against the toxic effect of oxidants [14].

In the present study, erythrocyte CAT activity was significantly decreased in smokers. The decrease in enzyme activity most probably reflects the increased oxidative stress thought to occur in smokers. This may be due to excess oxygen free radicals and other free radicals which oxidize, decompose and destroy the –SH group of antioxidant constituents, thereby weakening their activities [15]. The catalase is widely distributed in all animal tissues and high activity is found in red blood cells. Catalase has been suggested to play an important role in the protection of the erythrocyte against oxidative stress [16]. This may be the reason of no significant differences in erythrocyte SOD and GPx activities between the smokers and non-smokers in present study.

Among smokers, erythrocyte CAT activity increased with the number of cigarettes consumed per day. This may explain that increase CAT activity exerts a protective effect from subsequent oxidative stress. From these findings, it can be hypothesized that reactive oxygen species (ROS) produced from cigarette smoking reduces the activity of erythrocyte CAT. However, when the ROS reach to a certain level by smoking, there is increased erythrocyte CAT activity probably due to induction of the enzyme. This needs to be elucidated by studying the CAT expression by various smoking status.

Reactive oxygen species can damage all types of biological molecules. Oxidative damages to proteins, lipids or DNA may all be seriously deleterious and may be concomitant [17]. However, proteins are possibly the most immediate vehicle for inflicting oxidative damage. Some studies

showed higher plasma carbonyl level in smokers than in non-smokers [18, 19, 20]. However, a study showed that there was a trend towards higher carbonyl concentration in smokers than that in controls but they were not significantly different. The plasma carbonyl concentration in smokers was significantly higher than that in controls after oxidation of the plasma [21].

One study found out that carbonyl levels were higher among current smokers, but these differences did not attain statistical significance, nor did differences by urine cotinine levels, pack-years, pack/ day among current smokers and smoking duration [22]. There was no significant difference in plasma carbonyl protein levels between smokers and non-smokers in the present study. This may be due to the fact that the proteolytic enzymes are degrading and eliminating the oxidatively altered proteins effectively, thus preventing accumulation of altered and damaged proteins.

It is noteworthy that the higher carbonyl protein levels was independently associated with increasing age [19, 21, 23]. Age related accumulation of carbonyl protein in different human tissues was studied [24] and it was found out that there was little or no change in the level of protein carbonyls during the first 45 years of life but increased abruptly in linear fashion after age of 60 years. Therefore, the apparent effect of smoking on plasma carbonyl protein level is more marked in elderly subjects.

In conclusion, cigarette smoke causes oxidative stress. One of oxidative defense enzymes, erythrocyte catalase activity, was significantly decreased in smokers aged between 25-40 years consuming 3-15 cigarettes per day. Among smokers, the catalase activity increased according to the numbers of cigarettes consumed per day and further studies need to investigate the pattern of catalase enzymes responses to the oxidants of cigarette smoke in various smoking status. Concerning the marker of oxidative damage, plasma carbonyl protein was not obviously affected by cigarette smoking in this study. This may be probably due to the effective proteolytic enzymes, relative young age in the present study population and relative less pack-years than that of the other previous studies.

ACKNOWLEDGEMENT

We would like to express our heartfelt gratitude to Dr. Khin Nwe Oo, Director (Research), Department of Medical Research (Lower Myanmar) for her help in chemical purchasing and Dr. Thida Aung, Associate Professor/Consultant Pathologist, National Blood Centre, Yangon for allowance to collect blood samples from the blood donors.

REFERENCES

1. WHO: Economic, Social and Health Issues in Tobacco Control. *Report of a WHO International Meeting, Kobe, Japan:* WHO 2003: 22.
2. Mathers CD & Loncer D. Projections of global mortality and burden of disease from 2002 to 2003. *The Public Library of Science and Medicine* 2006; 3(11):2011-2030.
3 Pryor WA & Stone K. Oxidants in cigarette smoke: radicals, hydrogen peroxides, peroxynitrate, and peroxynitrite. *Annals of the New York Academy of Sciences* 1993; 686: 12-28.
4. Michels C, Raes M, Toussaint O & Remacle J. Importance of Se-glutathione peroxidase, catalase, and Cu/Zn SOD for cell survival against oxidative stress. *Free Radical Biology and Medicine* 1994; 17: 235-248.
5. Sohn HO, Lim HB, Lee YG, Lee DW & Kim YT. Effect of sub-chronic administration of antioxidants against cigarette smoke exposure in rats. *Archives of Toxicology* 1993; 67:667-73.
6. Berlett BS & Stadtman ER. Protein oxidation in aging, disease, and oxidative stress. *The Journal of Biological Chemistry* 1997; 272 (33): 20313-20316.
7. Reznick AZ, Cross CE, Hu ML *et al*. Modification of plasma proteins by cigarette smoke as measured by protein carbonyl formation. *Biochemistry Journal* 1992; 286 (Part 2): 607-611.
8. WHO: *Guidelines for Controlling and Monitoring the Tobacco Epidemic.* Geneva, WHO 1998: 76-77.
9. Drabkin DL. Determination of hemoglobin by cyanmethemoglobin method. *The Journal of Biological Chemistry* 1946; 164: 703-723.

10. Aebi H. Catalase *in vitro*. *Methods in Enzymology* 1984; 105: 121-126.
11. Takahashi K. Glutathione peroxidase: coupled enzyme assay. In: *Experimental Protocols for Reactive Oxygen and Nitrogen Species*. Taniduchi N and Gutteridge JMC (eds), Academic Press, London, 2000; 79-80.
12. Suzuki K. Measurement of Mn-SOD and Cu,Zn-SOD. In: *Experimental Protocols for Reactive Oxygen and Nitrogen Species*. Taniduchi N and Gutteridge JMC (eds), Academic Press, London, 2000; 91-95.
13. Reznick AZ & Packer L. Oxidative damage to proteins: Spectrophotometric method for carbonyl assay. *Methods in Enzymology* 1994; 233: 357-363.
14. Brown KM, Morrice PC, Arthur JP & Duthie GG. Effects of vitamin E supplementation on erythrocyte antioxidant defense mechanisms of smoking and non-smoking men. *Clinical Science* 1996; 91:107-11.
15. Ortega RM, Lopez-Sobaler AM & Quintas ME, Martinez RM & Andres P. The influence of smoking on vitamin C status during the third trimester of pregnancy and on vitamin C levels in maternal milk. *Journal of the American College of Nutrition* 1998; 17(4), 379-384.
16. Scott MD, Lubin BH, Zuo L & Kuypers FA. Erythrocyte defense against hydrogen peroxide pre-eminent importance of catalase. *Journal of Laboratory Clinical Medicine* 1991;118 (1): 7-16.
17. Bast A, Haenen Guido RMM & Doelman CJA. Oxidants and antioxidants: State of art. *The American Journal of Medicine* 1991; 91: 2S-22S.
18. Lee BM, Lee SK & Kim HS. Inhibition of oxidative DNA damage, 8-OHdG, and carbonyl contents in smokers treated with antioxidants (vitamin E, vitamin C, β-carotene and red ginseng). *Cancer Letters* 1998; 132: 219-227.
19. Pignatelli B, Li CQ, Boffetta P *et al*. Nitrated and oxidized plasma protein in smokers and lung cancer patients. *Cancer Research* 2001; 61: 778-784.
20. Hemalatha A, Venkatesan A, Bobby Z, Selvaraj N & Sathiyapriya V. Antioxidant response to oxidative stress induced by smoking. *Indian Journal of Physiology and Pharmacology* 2006; 50 (4): 416-420.
21. Marangon K, Devaraj S & Jialal I. Measurement of protein carbonyls in plasma of smokers and in oxidized LDL by an ELISA. *Clinical Chemistry* 1999; 45 (4): 577-578.
22. Yeh CC, Barr RG, Powell CA *et al*. No effect of cigarette smoking dose on oxidized plasma proteins. *Environmental Research* 2008; 106: 219-225.
23. May Pyone Kyaw. Effects of antioxidant vitamins on age related changes in markers of oxidative stress in apparently healthy elderly individuals. *Thesis for the Degree of Doctor of Philosophy of Medical Science in Biochemistry*. University of Medicine 2, 2005.
24. Moskovitz J, Yim MB & Chock PB. Free radicals and disease. *Archives of Biochemistry and Biophysics* 2002; 397(2): 354-359.

The Myanmar Health Sciences Research Journal, Vol. 22, No. 1, 2010

Detection of brucellosis in animal handlers and raw milk of selected dairy cattle farms in Yangon

**Thuzar Myint, **Win Maw Tun, *Wah Wah Aung, ***Zin Mar Aung, *Myat Thida,*Aye Aye Maw, *Aye Yin Shwe & ****Khin Nwe Oo*

*Bacteriology Research Division
**Experimental Research Division
****Department of Medical Research (Lower Myanmar)
*** Livestock Breeding and Veterinary Department

Brucellosis is an important zoonotic disease and an occupational hazard. A cross-sectional field survey was carried out in dairy cattle farms in Hlegu and Taikkyi townships, Yangon, from January to July 2009 to detect brucella infection in people who were exposed to animals and in raw milk. A total of 150 blood samples (75 animal handlers and 75 controls) were tested to detect IgG antibody to *Brucella* spp. by using *Brucella* IgG ELISA test and 80 raw milk specimens from dairy cattle of study areas were tested to identify *Brucella* spp. by culture. *Brucella* IgG was detected in 8 (10.7%) animal handlers and not detected in controls. *Brucella* spp. was isolated from 4 (5%) raw milk samples. The occurrence of bovine brucellosis and serological evidence of brucella infection among the animal handlers in the study areas indicated the need of large scale study to reveal the magnitude of human brucellosis among occupationally exposed people in Myanmar.

INTRODUCTION

Brucellosis is an important zoonotic disease and an occupational hazard. The infection can be transmitted directly and indirectly to dairy workers, shepherds, veterinarians, abattoirs and animal husbandry workers. The common causal bacterial organism, *Brucella abortus* (primary reservoir in cattle) is shed in milk, urine and vaginal discharges of the animals which can contaminate the environment. The mode of infection is through ingestion of unboiled milk of infected cattle, contact with vaginal discharges, urine, faeces and blood of infected animals, through unbreached skin and mucous membrane of conjunctiva and by inhalation. Although brucella infections are widely distributed in both human and animals in the developing countries, mildness and chronicity of the disease makes the diagnosis difficult [1].

Persons infected with *Brucella* spp. usually have sign and symptoms consistent with influenza-like or septicemic illness-like undulating fever, fatigue, malaise, chills, sweating, myalgia, arthralgia and weight loss. There are many reports on the incidence of human brucellosis in India. The incidence of brucellosis was 8.5% in animal handlers in and around Burla [2]. In one study, 11 out of 20 brucellosis cases were connected with animal husbandry [3]. Brucellosis has emerged as a major public health concern in Saudi Arabia and Middle East countries as 20% of the population had serological evidence of exposure [4].

Myanmar is an agricultural country and exposure to animals is quite high. A report of the first international conference on emerging zoonoses in Jerusalem described that bovine brucellosis and porcine brucellosis cases were found in Myanmar in 1994 [5]. There are very few studies of

brucellosis undertaken in an occupationally exposed group. Hence, the present study was conducted to assess the prevalence of brucellosis in high-risk occupational group and in raw milk (unpasteurized milk) in dairy cattle farms in Yangon, Myanmar.

General objective

- To determine the prevalence of brucellosis in people who are exposed to animals and in raw milk

Specific objectives

- To detect the IgG antibodies of *Brucella* spp. by ELISA test in animal handlers in selected cattle farms in Hlegu and Taikkyi townships, Yangon
- To identify *Brucella* spp. from raw milk (unpasteurized milk) from dairy cattle of above-mentioned dairy cattle farms

MATERIAL AND METHODS

A cross-sectional, field and laboratory-based descriptive study from January to July 2009 in dairy cattle farms of Hlegu and Taikkyi townships, Yangon

Study population and sample size

Seventy-five animal handlers including 20 dairy cattle farm workers from Hlegu, 50 dairy cattle farm workers from Taikkyi and 5 veterinary surgeons and 80 raw milk samples of dairy cattle (43 samples from Hlegu and 37 samples from Taikkyi) in study area. Seventy-five healthy individuals (>18 years) who were involved in an occu-pation other than veterinary services and animal husbandry served as the control group.

Subject selection

- Aged 18 years and above, both sexes
- Those who work in dairy cattle farms for at least 6 months and the nature of the job is related with handling of cattle, their excreta and/ or processing of raw milk
- Those who gave written informed consents

Sample collection

After taking the informed consents, demographic and clinical data were collected from eligible participants. Five milliliter of venous blood specimens from animal handlers were collected by vene-puncture under aseptic condition. Fifty milliliters of unpasteurized (raw) milk from cows were collected directly to sterile bottles at the field sites and kept in ice boxes. They were sent to Bacteriology Research Division to perform laboratory procedures.

Laboratory procedures

Brucellosis is diagnosed according to WHO case definition and laboratory criteria [6]:

- Isolation of *Brucella* spp. from clinical specimens, or
- ELISA (IgA, IgG, IgM) test positive

Detection of Brucella IgG antibodies

From the blood samples, serum was separated by centrifugation. Serum speci-mens were kept in –20˚C. Detection of *Brucella* IgG antibodies was done by using *Brucella* IgG ELISA test kit, Panbio Diagnostics, Australia, on test and control samples according to manufacturer's instruction [7,8].

Identification of Brucella spp. in raw milk

The raw milk samples were centrifuged for 5 minutes at 2000 rpm. Precipitates were inoculated onto Brucella agar and blood agar and incubated at 35-37˚Ċ for 7 days. Gram staining, oxidase test, urease test and serotyping were performed on suspected iso-lates to confirm the aetiological diagnosis [9].

Ethical consideration

This study was approved by the Institutional Ethical Review Committee of Department of Medical Research (LM) by the letter no. 23/Ethics 2007 on 21st February 2008.

RESULTS

Prevalence of Brucella IgG antibodies in animal handlers

Out of 75 animal handlers, including 5 dairy farm workers and 3 veterinary surgeons 8 (10.7%) showed presence of *Brucella* IgG antibody. Distribution of detected cases among animal handlers is shown in Table 1.

Table 1. Distribution of *Brucella* IgG seropositive cases among animal handlers

Animal handlers	Tested cases (n)	No. of *Brucella* IgG positive cases		Total positive cases (%)
		Male	Female	
Dairy farm workers in Hlegu	20	4	-	4 (20)
Dairy farm workers in Taikkyi	50	-	1	1 (2)
Veterinary surgeons	5	1	2	3 (60)
Total	75	5	3	8 (10.7)

Demographic and clinical data of Brucella seropositive persons

The demographic data and clinical data of 8 *Brucella* seropositive persons are shown in Table 2.

Table 2. The demographic and clinical data of 8 *Brucella* seropositive persons

Demographic data	Total tested cases	Total infected cases	%
Age (years)		21-58	
Sex			
Male	37	6	16.2
Female	38	2	5.30
Nature of work			
Dairy farm work	70	5	7.1
Feeding	16	1	6.3
Milking and feeding	32	4	12.5
Cleaning	14	-	-
Bottling of milk	8	-	-
Veterinary surgeon	5	3	60.0
Duration of work (in contact with animals)			
<5 years	27	1	3.7
>10 years	48	7	14.6
History of consuming raw milk	Nil		
Relevant past clinical history			
Presence of symptoms like (fever, joint pain, headache, malaise, myalgia etc.)	36	8	22.2
No symptoms	39	0	

Table 3. Distribution of *Brucella* spp. culture positive cases in Hlegu and Taikkyi Townships

Study site	Tested milk samples	No. of culture-positive samples	Percentage of culture-positive samples
Hlegu	43	3	6.9
Taikkyi	37	1	2.7
Total	80	4	5

Identification of Brucella spp in raw milk

Out of 80 raw milk samples, *Brucella* spp. was identified in 3 samples from Hlegu and 1 sample from Taikkyi (Table 3). Cows of all culture-positive milk samples were also positive for *Brcella* IgG by testing with in-house ELISA method which was carried out at Research and Biologics Subdivision of Livestock Breeding and Veterinary Department. All of them had history of abortion, retained placenta, decreased conception rate and infertile.

DISCUSSION

Out of 75 animal handlers, 8 (10.7%) had brucella antibodies in their serum. In our study, 7.1% of dairy farm workers (5 out of 70) showed presence of brucella antibodies. This is slightly higher than the 4% seropositivity rate of dairy workers in India and lower than 31.82% seropositivity in livestock workers in Nigeria [10, 11]. Dairy farm workers are constantly exposed to infection due to contamination of hands and arms while milking the animals. The dairy farm workers also spend a considerable amount of time tending to the animals needs and attending to them in parturition and often sleeping alongside them. None of study participants gave history of drinking raw milk.

Sixty percent of veterinary doctors (3 out of 5) showed presence of antibodies to *Brucella* spp. The veterinary doctors are exposed to all kinds of risk run by dairy farmers except that they are aware of the disease. They contract the disease by contamination of the skin or conjunctiva or

by accidental inoculation of live cultures. They incur additional risk in obstetric practice in removal of retained placenta and uterine products. There was a seropositivity of 27.7% in veterinary workers in Delhi and 14.63% seopositivity in veterinary surgeons in Pune [12, 13]. In our study, only five veterinary surgeons were included, thus further large scale study focusing on that population is suggested to reflect the actual prevalence among the veterinary surgeons.

Brucellosis has protean manifestations with complaints ranging from arthralgia, arthritis, myalgia and intermittent fever which get diagnosed as pyrexia of unknown origin (PUO). In the present study, although acute brucellosis cases cannot be revealed, all sero-positive patients had past history of having symptoms like fever, malaise, joint pain, arthralgia and myalgia. Seven out of 8 seropositive cases were seen in people who gave history of contact with the cattle for more than 5 years.

Two out of three seropositive veterinary doctors had four years of service in the veterinary laboratory concerning with production of brucella vaccine. As brucellosis is the most commonly reported laboratory-associated infection, there is a need to conduct active surveillance of development of febrile illness or other signs of brucellosis among the laboratory workers exposed to brucella isolates.

In the present study, *Brucella* spp. was isolated from 4 samples of raw milk and all infected cows gave no history of brucella vaccination. Four sero-positive human cases were dairy farm workers from the cattle farm in Hlegu. According to the data from Livestock Breeding and Veterinary Department, the cattle from that farm gave history of bovine brucellosis during 2008. Thus, there may be possible zoonotic transmission of brucellosis in that area.

The clinicians miss many cases of brucellosis because it is not considered as an alternative diagnosis. The clinician should keep in mind the possibility of an occupational or environmental exposure in cases of pyrexia of unknown origin. It would also be worthwhile to create awareness of the disease in such professions so that necessary precautions and periodic screening of such occupationally exposed people can be done.

According to a report of the first international conference on emerging zoonoses in Jerusalem, bovine brucellosis and porcine brucellosis cases were found in Myanmar in 1994. As there is limited data of human brucellosis in Myanmar, studies are needed not only to assess the morbidity but also on preventive aspect which focuses on elimination of infection in cattle along with hygiene, vaccine, and effective heating and pasteurization of dairy products and related foods.

REFERENCES

1. Young EJ. Human brucellosis. *Review of Infectious Diseases* 1983; 5: 821- 842.
2. Mahakur TN. A study of human brucellosis based on cultures isolated from man and animals. *Indian Journal of Medical Research* 1968; 56: 250- 258.
3. Koshi G & Meyers RM. Report on twenty bacteriologically diagnosed cases of brucellosis encountered in routine laboratory service over a period of ten years. *Indian Journal of Medical Research* 1988; 88: 322- 329.
4. Cooper CW. The epidemiology of human brucellosis in a well defined urban population in Saudi Arabia. *Tropical Medicine and Hygiene* 1991; 94: 416- 422.
5. First Internal Conference on Emerging Zoonoses, Jerusasalem, Israel. Brucellosis: an over-view. *Emerging Infectious Diseases* 1997; 3(2):213-220.
6. Surveillence of human brucellosis: Available from URL: http://www.fao.org/docrep/ 006/ Y4723E/y4723e06.htm.
7. Voller A, Bartlett B & Bidwell DE. Enzyme immunoassays with special reference to ELISA techniques. *Journal of Clinical Pathology* 1978; 31: 507- 520.
8. Instruction Manual: Brucella IgG ELISA Cat. No. E-BAB01G received 09.01.2009, Panbio diagnostics.
9. Weyant RS, Popovic T, Bragg S, Lindsey KQ & Morse SA. Basic Laboratory protocols for

the presumptive identification of Brucella species CDC. C*enters for disease control and prevention* 2001; 1- 11.

10. Soman S & Kothari G. Human brucellosis. *Journal of Indian Medical Association* 1954; 24: 44-48.
11. Cadmus SIB, Ijagbone IF, Oputa HE, Adesokan HK & Stack JA. Serological survey of brucellosis in livestock animals and workers in Ibadan, Nigeria. *African Journal of Biomedical Research* 2006; 9:163-168.
12. Rana UVS, Sehgal S & Bhardwaj M. A sero-epidemiological study of brucellosis among workers of veterinary hospitals and slaughter house of Union Territory of Delhi. *International Zoonoses* 1985; 12: 74-79.
13. Mudaliar S, Bhore A & Pandit D. Detection of antibodies to *Brucella abortus* in animal handlers. *Indian Journal of Medical Sciences* 2003;57(3):181-186.

The Myanmar Health Sciences Research Journal, Vol. 22, No. 1, 2010

Patterns of anti-tuberculosis drug resistance among HIV patients with pulmonary tuberculosis attending the Specialist Hospital, Waibargi, Yangon

Wah Wah Aung, **Sabai Phyu,Htin Aung Saw, ***Ti Ti, *Phyu Win Ei & **Rai Mra*

*Bacteriology Research Division
Department of Medical Research (Lower Myanmar)
**Clinical Research Unit, HIV/AIDS, Specialist Hospital, Waibargi
***National Tuberculosis Program, Myanmar

The emergence of multi-drug resistant tuberculosis (MDR-TB), TB that exhibits resistance to two potent first-line anti-TB drugs, rifampicin and isoniazid, becomes not only a threat to the global TB control program but also a particular concern among HIV-infected persons. A cross-sectional descriptive study was carried out from November 2007 to January 2009 to determine the magnitude and patterns of anti-TB drug resistance among HIV-TB co-infected patients at the Specialist Hospital, Waibargi, Yangon. A total of 44 *Mycobacterium tuberculosis* culture isolates of HIV patients with pulmonary tuberculosis were tested for susceptibility to streptomycin, isoniazid, rifampicin and ethambutol at National Reference TB Laboratory. Overall resistance to any anti-TB drugs was found in 68.2% (30/44) of isolates. MDR-TB was found in 65.9% (29/44) of isolates. New cases of TB accounted for 31.8% (14/44) of the study group. Drug resistance in this subgroup was 35.7% (5/14) compared to 83.3% (25/30) in previously treated subgroup. Twenty-nine MDR-TB isolates were further tested for susceptibility to pyrazinamide and its resistance was found in 41.4% (12/29) of isolates. Those who had history of previous anti-TB treatment and those with $CD4^+$ count of less than 100 cells/µl were significantly more likely to suffer from drug resistance than those without (Crude OR=9, 95% CI=2.1-28.3, P=0.004; Crude OR=4, 95% CI=1.1-15.4, P=0.074, respectively). This study highlighted the magnitude of MDR-TB among HIV patients and the importance of effective management program.

I NTRODUCTION

Tuberculosis (TB) remains globally an important cause of morbidity and mortality. Almost one third of the world's population has been infected with *Mycobacterium tuberculosis*, the causative agent. Approximately 9 million new cases occurred in 2006 and more than 1.5 million deaths were attributed to TB [1]. Human immunodeficiency virus (HIV) had also a great impact on health with nearly 32 million individuals currently infected worldwide [1]. Tuberculosis is the commonest opportunistic infection among people infected with HIV in developing countries and it is seen in 70% to 80% of the cases [2].

The emergence of multi-drug resistant TB (MDR-TB) that exhibits resistance to two potent first-line anti-TB drugs, rifampicin and isoniazid, not only threatens the global TB control but also becomes a particular concern among HIV-infected persons. MDR-TB can lead to poor prognosis and rapid disease progression which may result in rapid transmission of resistant bacilli to other immunosupressed patients [3]. Approximately half a million cases of MDR-TB were observed globally in 2006. In Myanmar, MDR-TB rate among new and previously treated TB patients in Yangon Divisional TB Centre were 4.4% and 16%, respectively, in 2004. HIV infections were found in 5% of TB patients [4].

MDR-TB has been shown to be almost twice as common in TB patients living with HIV compared to TB patients without HIV [1]. Although there are contradictory reports on the associations of HIV infection and anti-TB drug resistance, high rates (43%, 35/81) of MDR-TB were documented among HIV-infected persons in public hospitals in Lima and Callao, Peru during 1999 to 2000 [5]. HIV-infected persons have a higher risk of acquisition of isolated rifampicin resistance [6]. The existence of malabsorption of rifampicin and isoniazid was shown in HIV-infected patients with or without diarrhoea [7]. There was also an increased frequency of rifampicin resistance among the patients having $CD4^+$ count of less than 100 cells/cu mm [8].

An even greater challenge and a serious health threat is the appearance of XDR-TB (Extensive Anti-TB Drug Resistance) which is MDR-TB that is also resistant to any fluoquinolone and at least one of three injectable anti-TB drugs (capreomycin, kanamycin and amikacin). A recent out-break of XDR-TB cases in South Africa has been found almost exclusively among the HIV patients and the mortality was found to be 85% [9].

The World Health Organization (WHO) provides guidance on recent developments in the approach to TB/HIV and recommends the HIV/MDR-TB activities. Since there is limited data on the anti-TB drug resistance status among HIV patients in Myanmar, the present study was, thus, carried out to explore the magnitude and pattern of anti-TB drug resistance among HIV patients with pulmonary tuberculosis attending the Specialist Hospital, Waibargi, Yangon and to find out the occurrence of MDR-TB cases among these patients.

MATERIALS AND METHODS

A cross-sectional descriptive study was carried out at the Specialist Hospital, Waibargi from November 2007 to January 2009.

Study population

HIV seropositive patients co-infected with active pulmonary tuberculosis (diagnosed on the basis of acid-fast bacilli seen in sputum smear or culture results or on clinical characteristics) attending the Specialist Hospital, Waibargi during the study period.

Inclusion criteria

- Aged 12 years and above, both sexes
- HIV seropositive patients presenting with active pulmonary tuberculosis (both new and previously treated TB, both sputum acid-fast bacilli smear positive and negative, both in-patient and out-patient)
- Those who gave written informed consent

Exclusion criteria

- Those who were too ill and cannot produce sputum
- Those with extra-pulmonary TB

Data collection

Socio-demographic and clinical data

After taking the informed consent, the relevant socio-demographic data and clinical data regarding the symptoms and duration of disease, previous history of anti-TB treatment and duration, concomitant diseases, history of HIV infection, investi-gations such as chest X rays, $CD4^+$ count, laboratory tests etc. were collected.

Laboratory procedures

Sample collection

Two sputum specimens were collected with clean, wide mouth, screw-capped containers. Then they were sent to the National Reference TB Laboratory in ambient temperature. If there is delay for more than 12 hours, they were kept cool in refrigerators (2˚C to 10˚C).

Sputum microscopy, culture and drug susceptibility testing

Sputum microscopy was carried out by Ziehl Neelsen method. Isolation of

M. tuberculosis was attempted in 112 patients, 54 showed growth on Lowenstein-Jensen (LJ) media at 37˚C for 6-8 weeks. Drug susceptibility testing was requested for all positive cultures and completed on 44 who were the focus of subsequent analyses. *Mycobacterium tuberculosis* isolates were identified by its growth rate and pigmentation. Drug susceptibility to first-line anti-TB drugs, isoniazid, rifampicin, streptomycin and ethambutol was carried out by proportion method [10]. Twenty-nine *M. tuberculosis* isolates showing resistance to isoniazid and rifampicin were further tested for pyrazinamide susceptibility on acidified LJ media.

Anti-TB drug resistant cases were categorized as follows according to the definition by WHO, 2008.

Drug resistance among new cases

Presence of resistant isolates of *M. tuberculosis* in patient who denied having any prior anti-TB treatment for less than one month in response to direct questioning.

Drug resistance among previously treated cases

Presence of resistant isolates of *M. tuberculosis* in patient who admitted having been treated for tuberculosis for one month or more in response to direct questioning.

Data analysis

Only 44 cases whose all laboratory results for drug susceptibility tests are available were considered in the analysis. The data were entered and analyzed by using SPSS for Windows version 11.5 software. The drug susceptible and resistant cases were documented as the percentages of the total study population and the drug resistance pattern of anti-TB drugs in the new and previously treated patients was analysed. Frequency distribution and cross tabulation of variables were constructed. Crude odds ratio and 95% confidence intervals were computed and p value ≤0.05 was considered as significant.

Ethical consideration

This study was approved by the Institutional Ethical Review Committee of Department of Medical Research (Lower Myanmar).

RESULTS

Demographic and clinical characteristics of HIV patients with pulmonary tuberculosis

The demographic data and relevant clinical characteristics of 44 HIV patients with pulmonary tuberculosis are shown in Table 1.

Table1. The demographic and clinical characteristics of HIV patients with pulmonary tuberculosis (n=44)

Demographic data		Number	%
Age	Mean age in years	34 ± 6.08 (Range- 23-51)	
Sex	Male	32	72.7
	Female	12	27.3
Marital status	Currently married	23	52.3
	Single	21	48.7
Occupation	Working group	14	31.8
	Dependent	30	68.2
Education	Read & write	8	18.2
	Primary school	11	25.0
	Middle school	12	27.3
	High school	12	27.3
	University	1	2.2
History of previous anti-TB treatment	New	14	31.8
	Previously treated	30	68.2
Present symptoms	Cough	44	100
	fever	35	79.5
	Wt loss	41	93.2
	Haemoptysis	2	4.5
	Chest pain	11	25.0
Associated infections	Herpes zoster	5	11.4
	Skin manifestations	14	31.8
	Penicillinosis	1	2.3
	Cryptococcosis	2	4.5
	Diarrhoea	15	34.1
	Genital ulcer	2	4.5
	Syphilis	4	9.1
Sputum smear acid fast bacillli	Positive	32	72.7
	Negative	12	27.3
Chest x'ray	Koch Lung	19	43.2
	Miliary TB	6	13.6
	Basal pneumonia or/ and pleural effusion	6	13.6
	No report	14	31.8
CD4 $^{+}$count	<100 cells/µl	31	70.5
	>100 cells/µl	13	29.5

Anti-TB drug resistance pattern

Drug susceptibility patterns of 44 *M. tuberculosis* isolates to first-line anti-TB drugs, isoniazid (H), rifampicin (R), streptomycin (S) and ethambutol (E) among HIV patients with new and previously treated pulmonary TB are shown in Table 2.

Table 2. First- line anti -TB drug resistance pattern among *M. tuberculosis* isolates from HIV patients with pulmonary tuberculosis (n=44)

Description	Previously treated cases n (%)	New cases n (%)	Total n (%)
Whole studied group	30 (68.2)	14 (31.8)	44 (100)
Sensitive to all drugs	5 (16.7)	9 (64.3)	14 (31.8)
Resistance to any drug	25 (83.3)	5 (35.7)	30 (68.2)
MDR-TB	25 (83.3)	4 (28.6)	29 (65.9)
S,H,R,E	16 (53.3)	1 (7.1)	17 (38.6)
S,H,R	4(13.3)	1 (7.1)	5 (11.3)
H,R,E	2(6.7)	-	2 (4.5)
H,R	3 (10.0)	2 (14.3)	5 (11.3)
One drug resistance			
S	-	1 (7.1)	1 (2.3)

H= isoniazid, R= Rifampicin, E= Ethambutol,
S= Streptomycin, MDR-TB= Multi-drug resistant TB

Only 14 (31.8%) isolates were sensitive to all tested drugs. Overall resistance to any anti-TB drug was documented in 30 (68.2%) isolates. MDR-TB was found in 29 (65.9%) isolates comprising of SHRE resistance in 17 (38.6%), SHR resistance in 5 (11.3%), HRE resistance in 2 (4.5%) and HR resistance in 5 (11.3%) isolates. One drug resistance, S resistance was seen in 1(2.3%) isolates. The total resistance to individual anti-TB drug was H (29, 65.9%), R (29, 65.9%), S (23, 52.3%) and E (18, 40.1%). Anti-TB drug resistance among new TB cases was 35.7% (5/14) compared to 83.3% (25/30) in previously treated subgroup.

Pyrazinamide resistance among MDR-TB patients

Of tested 29 MDR-TB isolates, pyrazimide (Z) resistance was found in 12 (41.4%) (Fig. 1) . Among them, 11 (37.9%) isolates were SHRE resistant isolates, thus these isolates showed resistance to all five first-line anti-TB drugs, S, H, R, E, and Z.

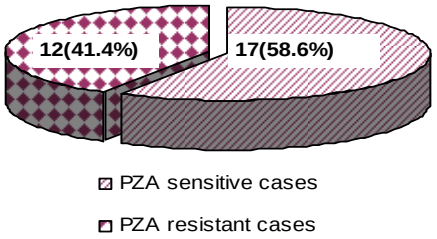


Fig.1 Pyrazinamide resistance among MDR-TB cases (n=29)

Anti-TB drug resistant cases among new and previously treated patients

HIV patients who had history of previous anti-TB treatment were significantly more likely to suffer from anti-TB drug resistance than those without. Out of 25 drug resistant patients who had previous anti-TB treatment history, 18 patients were defaulters. Distribution of drug resistant cases among new and previously treated group is shown in Table 3.

Table 3. Drug resistant TB in relation to history of previously anti-TB treatment

Previous treatment for TB	Anti-TB drug resistant cases n (%)	Anti-TB drug sensitive cases n (%)	Total n (%)
Yes	25 (83.3)	5 (35.7)	30 (68.2)
No	5 (16.7)	9 (64.3)	14 (31.8)
Total	30 (100)	14 (100)	44 (100)

Crude OR=9, 95% CI=2.1-28.3, P=0.004

Distribution of anti-TB drug resistant cases in relation to $CD4^+$ count

The HIV patients who had $CD4^+$ count of less than 100 cells/µl were significantly more likely to suffer from anti-TB drug resistance than those who had $CD4^+$ count of more than 100 cells/µl. Distribution of anti-TB drug resistant cases in relation to $CD4^+$ count is shown in Table 4.

Table 4 . Drug resistant TB in relation to $CD4^+$ count

$CD4^+$ count	Anti-TB drug cases		Total n (%)
	resistant n (%)	sensitive n (%)	
<100 cells/µl	24 (80.0)	7 (50.0)	31 (70.1)
>100 cells/µl	6 (20.0)	7 (50.0)	13 (29.6)
Total	30 (100)	14 (100)	44 (100)

Crude OR=4, 95% CI=1.1-15.4, P=0.074

DISCUSSION

Surveillance of drug resistant TB plays an important role in determining the magnitude and trends. Managing the MDR-TB cases that emerge is part of the STOP TB strategy and a component of all TB Control Programmes. In addition, there is a need for a better understanding of epidemiological relationship between drug resistance and HIV infection [1]. This study provides on updated information using a selected group of HIV patients with pulmonary TB in a tertiary care hospital.

Overall resistance to any anti-TB drug and MDR-TB rates of HIV patients with pulmonary TB were 68.2% and 65.9%, respectively. This figure represents combined proportion of drug resistance which is the proportion of resistance in the population surveyed regardless of prior treatment. The total resistance to individual anti-TB drug was H (65.9%), R (65.9%), S (52.3%) and E (40.1%), respectively.

The anti-TB drug resistance pattern of the present study was a little higher than that of the study conducted in Waibargi Hospital revealing anti-TB drug resistance rate of 57.6% and MDR-TB rate of 50% [11]. A study in Mumbai, India also reported high resistance rate of 92% H, 65% R, 81% S and 77% E among *M. tuberculosis* isolates from HIV/TB co-infected patients [12]. Both studies were conducted on small number (<30) of *M. tuberculosis* isolates.

Previous studies on TB patients with unknown HIV status in Myanmar reported that the most common resistant drug was isoniazid and the second was streptomycin. In the present study, both isoniazid and rifampicin were the commonnest in drug resistance followed by streptomycin and ethambutol. One factor that could be responsible for rifampicin resistance is the malabsorption of anti-TB drugs in patients with advanced HIV infection which can lead to low blood levels. High levels of isoniazid resistance in the present study pointed out that the introduction of isoniazid preventive therapy for future TB and HIV control should be taken into consideration.

Drug resistance in new cases is used to evaluate the recent transmission and in previously treated group can be used as a proxy for acquired resistance. New cases of TB accounted for 31.8% (14/44) in the present study. Drug resistance in this subgroup was 35.7% (5/14) compared to 83.3% (25/30) in previously treated subgroup. A study reported the pattern of anti-TB drug resistance among 111 HIV patients in Maputo, Mozambique. Overall observed drug resistance was 18% and MDR-TB was 9% among new TB patients. Previously treated patients had high drug resistance, 26.3% and MDR-TB, 15.8% [13]. A prospective cohort study on 204 HIV/TB patients in India also reported 13% H resistance and 4.2% MDR-TB among new TB cases and 15-28% H resistance and 2.8-3.4% among previously treated TB patients [14].

The anti-TB drug resistance rate among new cases and previously treated cases reported by the present study is much higher than that of other studies conducted on large number of *M. tuberculosis* isolates. This may be due to rather small study population and/or selected patients were from the reference hospital to which most complicated cases were referred. Thus, in the present study, combined proportion of drug resistance may represent a better approximation of drug resistance in the selected community than separate data for new and previously treated patients.

In 2002-03 nationwide survey on anti-TB drug resistance among TB patients with unknown HIV status in Myanmar, resistance to any drug among new cases was 10% and among the previously treated group was 30.2% [4]. This finding indicated that the rate and pattern of anti-TB drug resistance might be different among HIV patients, thus large scale community-based studies are needed to determine the magnitude of anti-TB drug resistance problem in the study population.

In the present study, resistance to four first-line anti-TB drugs (SHRE resistance) was found in 17 (38.6%) patients in the study population and there was also high rate of five anti-TB drug resistance (SHREZ resistance). Moreover, to the best of our knowledge, this is the first study describing the pyrazinamide resistance pattern in Myanmar although we can only perform the laboratory test for pyrazinamide resistance on 29 MDR-TB isolates due to technical constraints.

It might be expected, those who had history of previous anti-TB treatment were 9 times significantly more likely to suffer from drug resistance than those without (Crude OR=9, 95% CI=2.1-28.3, P=0.004). MDR-TB is also higher in patients with previous anti-TB treatment. This finding is comparable to other studies conducted on non-HIV-infected population. Patients who had repeated attacks of TB and who had incomplete treatment or defaulting treatment were more likely to suffer from MDR-TB [15,16]. Our study also showed that out of 25 drug resistant patients who had previous anti-TB treatment history, 18 (72%) patients were defaulters.

The present study also pointed that HIV patients with $CD4^+$ count of less than 100 cells/µl were 4 times significantly more likely to suffer from anti-TB drug resistance than those with $CD4^+$ count of more than 100 cells/µl (Crude OR=4, 95% CI=1.1-15.4, P=0.074). HIV patients with TB had a reduced enrichment and activation of immune cells in the lung, reducing $CD4^+$ alveolitis as an effective immune response to acid-fast baccili, explaining the more severe TB cases, the need of re-treatment and probably the high frequency of drug resistance in low $CD4^+$ level patients. An interesting finding is that none of the drug resistant patients presented $CD4^+$ counts of more than 300 cells/µl.

Due to the limitation in this study, no outcome data at the end of treatment can be shown. Nevertheless this study showed a relatively high number of deaths (22 expired cases) among 29 MDR-TB patients (75.9%).

Another factor that contributed to the association of MDR-TB and HIV infection was that TB in HIV-infected persons might not be as easily recognizable as that in persons with normal immune response. For this reason, TB was unrecognized in some of those patients for prolonged periods and resulted in delayed institution for treatment. Finally, prolonged infectivity, despite anti-TB therapy, was also an important factor in the spread of MDR-TB.

Increased use of short course treatment regimen DOTs particularly in areas of high HIV prevalence may help overcome the emergence of drug resistance among HIV patients. Ensuring compliance during treatment may reduce the number of re-treatment cases and subsequently the drug resistance.

Conclusion

Overall anti-TB drug resistance and MDR-TB in HIV patients were high in this study. MDR-TB was associated with previous antiTB treatment and $CD4^+$ count of <100 cells/µl. Our results cannot be generalized to the whole country, not for all HIV patients with TB, due to possible selection bias. We selected patients in reference hospitals to which most compli-cated cases were referred. However, the data showed here represented a pattern of drug resistance indicating the importance of MDR-TB treatment programme. There is a need for better epidemiological surveillance, strict reinforcement of DOTS and collaboration between AIDS and TB control programme.

ACKNOWLEDGEMENT

The authors thank the Medical Superintendent, Dr. Khin Mar Nwe, Consultant Microbiologist and staff of Specialist Hospital, Waibargi for their coorperation in the study. The authors also thank Dr. Khin Thet Wai, DMR (LM) for statistical analysis.

REFERENCES

1. WHO. Anti-tuberculosis drug resistance in the world, fourth global report (2008). WHO/ HTM/ MTB/2008.394
2. Raviglione MC, Narain JP & Kochi A. HIV-associated tuberculosis in developing countries: clinical features, diagnosis, and treatment. *WHO Bulletin* 1992; 70:515-525.
3. MDR-TB Fact Sheet. Available from URL: http://www. who. int/ tb/ xdr/ faqs /en/ index. html access on 12/7/07.
4. Ti Ti, Thandar Lwin, Sabai Phyu, Wah Wah Aung, *et al.* Magnitude of the MDR-TB problem among patients attending Yangon Divisional TB Centre, Myanmar. *Myanmar medical Journal* 2007; 50(1): 2-7.
5. Campos PE, Suarez PG, Sanchez J, Zavala D, *et al.* Multi-drug resistant *Mycobacterium tuberculosis* in HIV-infected persons, Peru. *Emerging Infectious Diseases* 2003; 9(12): 1571-1577.
6. Nolan CM, Willams DL, Cave MD, *et al.* Evolution of rifampicin resistance in human immunodeficiency virus associated tuberculosis. *American Journal of Respiratory Critical Care Medicine* 1995; 152(3):1067-1071.
7. Gurumurthy P, Ramachandran G, Hemanth Kumar AK, Rajasekaran S *et al.* Malabsorption of rifampicin and isoniazid in HIV-infected patients with and without tuberculosis. *Clinical Infectious Diseases* 2004; 38: 280-283.
8. Rai Mra, Htin Aung Saw & Htun Lwin Nyein. Symposium on "New Down and New Hope, Q&A on ART" at 51st Myanmar Medical Conference, Mandalay, 2005.
9. HIV and tuberculosis fact sheet. Available from URL: http://www.lungusa.org/site/pp.asp: access on 21/9/07.
10. Venkatareman P & Paramasivan CN. Bacteriological methods in laboratory diagnosis of tuberculosis. Tuberculosis Research Centre (ICMR) Chetput, Chennei 600031, 1999.
11. Khin-Zaw-Latt. A study of tuberculosis among HIV/AIDS patients in Specialist Hospital (Waibergi). M Med Sc (Microbiology) thesis submitted to University of Medicine (1), 2005.
12. Birdi TZ, Dholakia Y, D'Souza DTB, Oza BB, *et al.* A preliminary report on initial Mycobacterial drug resistance in HIV-TB dual infection in Mumbai. *British Medical Journal* 2002; 18: 879-881.
13. Nunes EA, De Capitani EM, Coelho E, Joaquim OA, *et al.* Patterns of anti-tuberculosis drug resistance among HIV-infected patients in Maputo, Mozambique, 2002-2003. *International Journal of Tuberculosis and Lung Diseases* 2005; 9(5): 494-500.
14. Swaminathan. S, Paramasivan CN, Ponnuraja C, Iliayas S, Rajasekaran S & Narayanan PN. Anti-tuberculosis drug resistance in patients with HIV and tuberculosis in South India. *International Journal of Tuberculosis and Lung Diseases* 2005*;* 9(8): 896-900.
15. Than Lwin, Ti Ti & Tin Nu Swe. Drug resistance pattern in sputum positive treatment failure cases admitted to Aung San TB hospital. *The Myanmar Health Science Research Journal* 1996; 8(20): 78-82.
16. Reechaipichitkul W. Multi-drug resistance at Srinagarind Hospital, Khon Kaen, Thailand. *South East Asian Journal of Tropical Medicine and Hygiene* 2002; 33(3):570-4.

The Myanmar Health Sciences Research Journal Vol. 22, No. 1, 2010

Knowledge, attitude, and prevention practice on avian influenza among housewives in Mingalar Taungnyunt Township, Yangon

Yae Chan, Han Win, Aung Thu, Sandar Kyi, Kyu Kyu San, Myat Myat Thu, Ni Ni Aye, Khin Moe Latt & Khin Myat Tun

Clinical Research Division
Department of Medical Research (Lower Myanmar)

Avian influenza is a major threat to human as well as to animal health and has received a great deal of attention in recent years. This cross-sectional study was carried out in MingalarTaungnyunt Township, Yangon Division in December, 2008 to assess the knowledge, attitude, and prevention practices of avian influenza among housewives. A total of 212 respondents were interviewed by using questionnaires. The mean age of participants was 41.15±12.02 years. Most respondents (73.1%) had high knowledge levels. Majority (90.6%) had good attitude and 79.7% had good prevention practices of avian influenza. However, knowledge about major signs and symptoms of avian influenza in poultry was limited. Among the housewives, 95.3% had heard about the avian influenza infection and the majority (78.8%) reported television and radio as their source of information. Housewives who received information were more likely to have higher knowledge level about avian influenza ($p<0.001$) than those who did not. The high knowledge level of avian influenza was strongly related to higher education status ($p<0.001$), positive attitude ($p<0.003$) and good prevention practices ($p<0.001$). But it was not related to age ($p=0.625$), income ($p=0.495$) and occupation ($p=0.447$).The results of this study suggested that more effective health education programs about avian influenza than at present should be provided through mass media such as television and radio.

INTRODUCTION

Avian influenza is a major threat to human as well as to animal health and has received a great deal of attention in recent years. Since the discovery of the highly pathogenic H5N1 strain of avian influenza in China in 1996, the virus has spread rapidly within Asia, Europe, and Africa [1]. Indeed, the presence of the virus has been confirmed in birds or humans in more than 55 countries [2]. Moreover, in 19 June, 2008, there were 385 human cases of avian influenza reported in 15 countries, resulting in 243 deaths [3]. In Myanmar, highly pathogenic avian influenza H5N1 has been confirmed since 2006. Moreover, a human case of avian influenza was reported in 2007 [4].

The principle mean of transmission of avian influenza to human is through direct and close contact with infected live poultry [5]. This virus can be transmitted if a person has direct contact with infected poultry or surfaces and objects contaminated by poultry droppings. Housewives in the family, who commonly have contact with poultry, are at high risk for avian influenza. They are at increased risk because of food handling and preparation of raw poultry meat and products. However, the concern about the seriousness of the disease and the virus which can be more easily transmissible among humans has led national and international agencies to take action both to prevent and prepare for the possibility of a pandemic. In addition, influenza in domestic birds causes huge economic losses, and

directly affects food security and livelihood of rural areas in developing countries. [1]

Studies done in laboratories suggested that some of the prescription medicines approved in the US should work in treating avian influenza infection in humans. However, influenza viruses can become resistant to these drugs, so these medications may not always work [6]. As there are no effective immunization and treatment yet, precaution is the best way to control the spread of this infection.

Thus, the level of knowledge, attitude and prevention practice about avian influenza among housewives play a crucial role in combating this disease.

General objective

To determine knowledge, attitudes, and preventive practices related to avian influenza among housewives in Mingalar Taungnyunt Township, Yangon

Specific objectives

- To identify the sources of information with regards to avian influenza infection
- To assess the knowledge, attitudes and preventive practices about avian influenza among housewives
- To find out the association between demographic variables and knowledge scores; knowledge and attitude scores; knowledge and practice scores regarding avian influenza

MATERIALS AND METHODS

Study area and population

A cross-sectional study was conducted in Mingalar Taungnyunt Township, Yangon Division in December 2008. Patheinnyunt Ward was purposely selected because it was situated near the wet market. Then, housewives between 18 and 60 years of age, who were willing to give consent, were recruited consecutively till the required sample size was accomplished. In this study, a total of 212 subjects were recruited.

Data collection

Informed consent was obtained from each respondent and then they were interviewed (face-to-face) individually by trained interviewers using pre-tested structured questionnaire.

The main part of the questionnaire consisted of demographic characteristics and KAP concerning avian influenza.

Knowledge of avian influenza referred to the understanding of concepts of avian influenza related to: causative agent, source of information, modes of transmission, signs and symptoms of avian influenza infection, and preventive measures against avian influenza. This section consisted of 18 items, each items was scored with 1 or 0 for correct or incorrect answer, respectively. Levels of knowledge were categorized as "high" and "low" (median knowledge score was 9, ranging between 0-18).

Attitudes toward avian influenza referred to the degree of positive or negative agreement with 6 items concerning the severity of avian influenza, eating behaviors, cooking raw poultry, and using personal protective measures. Five point liker scales was used: "strongly agreed", "agreed", "don't know", "disagreed", and "strongly disagreed". Each item was scored with 5, 4, 3, 2, 1, or 0, respectively. The level of attitude scores was grouped into two categories as "positive" and "negative" (median score was 15, 0-30).

Practice related to avian influenza This section consisted of 8 items on avian influenza related preventive behaviors as general practice, and cooking practice. Each item was scored as 1 or 0 for correct or incorrect answer, respectively. Levels of practice were categorized as "good" and "poor" (median score was 4, 0-8).

Data analysis

Data were checked daily for error, incompleteness and consistency. Data entry was done by Epi data 4.0 and range and consistency check was carried out before

analysis. Data analysis was performed with SPSS 13.0 software. Univariate analysis was used to show the frequency distributions for categorical data and mean±SD for continuous data. The chi-square test was used to examine the $p \leq 0.05$ was considered significant association between categorical variables.

Ethical consideration

This study was approved by the Institutional Ethical Review Committee, Department of Medical Research (Lower Myanmar).

RESULTS

Characteristics of the study population

The overall mean age was 41.15±12.02 years. Regarding the education status, illiterates, those who can read and write, primary school passed, middle school passed, high school passed and graduated were, 4.2%, 6.6%, 20.8%, 31.1%, 25.5% and 11.8%, respectively. About half of the respondents were dependents (54.2%), followed by odd jobs (15.6%) and government staff (15.1%).

Table 1. Background characteristics (n = 212)

Variables	Number (%)
Mean age	41.15±12.02 years
Education status	
Illiterate	9 (4.2)
Can read & write	14 (6.6)
Primary school passed	44 (20.8)
Middle school passed	66 (31.1)
High school passed	54 (25.5)
Graduated	25 (11.8)
Occupation status	
Dependent	115 (54.2)
Odd jobs	33 (15.6)
Government staff	32 (15.1)
Non-government staff	8 (3.8)
Retailer	16 (7.5)
Own business	8 (3.8)
Marital status	
Single	35 (16.5)
Married	147 (69.3)
Divorced/ separated/widow	30 (14.2)
Median family size	5 (1-14)
Median family income per month (Kyat)	60,000 (7,000 - 500,000)

Majority of the respondents (69.3%) were married, 16.5% were single and the rest (14.2%) were divorced/ separated/widow. The median family size was 5 persons (range 1-14). The median family income was 60,000 kyats/month (range 7,000-500,000 kyats/month) (Table 1).

Source of knowledge information

Nearly all the respondents (95.3%) had heard about avian influenza infection. The majority (78.8%) reported television and/or radio as their source of information. The housewives who received information were more likely to have better knowledge level about avian influenza than those who did not (153/155, 98.7 %) ($p<0.001$).

Overall knowledge, attitude and prevention practice about avian influenza

Most of the respondents (73.1%) had high levels of knowledge. Among the study subjects, 90.6% had good attitude and most of the housewives (79.7%) had good prevention practices related to avian influenza (Table 2).

Table 2. Knowledge, attitudes and prevention practices (KAP) about avian influenza among housewives (n=212)

KAP levels	Number	%
Knowledge score (Total score = 18)		
Low (0-9)	57	26.9
High (10-18)	155	73.1
Attitude (Total score = 30)		
Poor (0-14)	20	9.4
Good (15-30)	192	90.6
Practice score (Total score = 8)		
Poor (0-4)	43	20.3
Good (5-8)	169	79.7

Knowledge about avian influenza

Nearly half of the housewives (48.6%) correctly answered the causal agent as avian influenza virus. Knowledge on modes of transmission of the disease varied: 90.6% knew that the disease could be transmitted from animal to animal, and 76.4% knew that the disease could be transmitted from animal to human. However, the

respondent's knowledge about major signs and symptoms of avian influenza in poultry was limited. Of the 212 housewives, 69.8% correctly responded that fever was a symptom of avian influenza infection and 48.6% responded correctly of sore throat. Other signs and symptoms such as breathing difficulty, diarrhea, muscle weakness and rashes were answered correctly by 42.5%, 37.7%, 32.5% and 32.1%, respectively.

Most of them (89.2%) knew to cook chicken and eggs at enough temperature and 87.7% knew to wash hands after handling live bird. Among the housewives, 86.8% knew proper disposal of carcasses and 84.4% answered correctly of washing chicken and egg before cooking. Nearly 81% knew to store raw poultry and other foods separately. More than two-third of housewives (77.4%) correctly indicated that sick or dead poultry should not be touched with bare hands and nearly two-third (72.6%) knew to avoid poultry farming inside or under the house.

Attitudes towards avian influenza

Majority (90.6%) of housewives strongly disagreed that one should bring dead chicken or poultry from the yard and prepare it to eat. A total of 89.2% of housewives agreed the importance of cooking raw poultry at adequate temperature and 88.7% worried about getting bird flu. Most of the housewives in this study (84.9%) disapproved to touch sick or dead poultry with bare hands and 83% of respondents agreed to touch with protective measures. However, more than half (53.3%) agreed to prepare raw poultry and other foods on the same cutting board after cleaning thoroughly and nearly half (44.8%) agreed that recent endemic flu can affect to all.

Preventive practices related to avian influenza

Regarding the cooking practice, 92.2% of housewives washed chicken and eggs before cooking. Most of the respondents (88.7%) cooked chicken and eggs at sufficiently high temperature and 72.6% did proper disposal of carcasses. More than half (53.8%) of the study participants, used the same cutting board after cleaning thoroughly. Among the participants, 40.1% did storage of raw poultry and other food separately. Regarding the general practice, most of the respondents (69.8%) avoided to go over-crowded places, and nearly half of the housewives took care about cleanliness (53.8%) and avoided contacting any live avian (50.5%), respectively. The age, family income and occupation of the housewives were not significantly related to knowledge score in bi-variate analysis (Table 3).

Table 3. Association between knowledge scores and demographic characteristics among housewives

Variables	Total knowledge score		χ^2	df	p value
	High	Low			
Age (n =212)					
Young (≤40 yrs)	50	48	0.24	1	0.625
Old (>40 yrs)	62	52			
Education (n=212)					
≤Middle school passed	14	86	43.82	1	0.0005
≥High school passed	65	47			
Income (n =212)					
Low (≤50,000 kyats/ month)	43	43	0.465	1	0.495
High (>50,000 kyats/month)	69	57			
Occupation group (n = 212)					
Dependent	58	57	0.579	1	0.447
Employed	54	43			

Association between knowledge, and demographic characteristics, attitudes, and preventive practices of avian influenza

The knowledge score was developed from combination of modes of transmission, sign and symptoms of bird flu, personal hygiene and control measures. Among housewives, 73.1% belonged to high knowledge group and the rest were in low knowledge group. The respondents who had finished middle school or above had higher percentages of avian influenza knowledge compared to

Table 4. Association between knowledge and attitude scores and knowledge and practice scores

Variables	Total knowledge score		χ^2	df	p value
	High	Low			
Attitude group (n=212)					
Poor	9	11	8.879	1	0.003
Good	146	46			
Practice group (n=212)					
Poor	5	38	36.75	1	0
Good	107	62			

those who had not passed middle school ($p<0.001$) (Table 3). The housewives who had positive attitude were more likely to have better knowledge level about avian influenza ($p=0.003$). There was a strong association with the housewives who had good preventive practices and high level of knowledge about avian influenza ($p<0.001$) (Table 4).

DISCUSSION

Just as the threat of an avian influenza pandemic is finally taken seriously by governments around the world, a small but increasing number of scientists are questioning how great the danger really is [8]. To the best of our knowledge, this is the first study to assess the knowledge of avian influenza among housewives in Myanmar.

In our study, there was a statistically significant association between housewives who received information about avian influenza through media (especially from television and radio) and higher knowledge level. These findings were consistent with the studies from Nigeria and Saudi Arabia. The results of the study in Nigeria showed that the source of information (television, radio and newspapers) was significantly associated with the overall knowledge score of poultry workers [7]. There was an association between knowledge score and source of information and the highest scores were observed among secondary school students in Saudi Arabia who reported that their sources of information were newspapers, parents, internet and media, respectively [8]. These studies suggested that this information helped people to learn about avian influenza infection and the TV program encouraged public awareness on how to prevent avian influenza infection [7, 8].

According to the results, more than two-third of the study population had correct knowledge, attitude and prevention practices about avian influenza. These findings were similar to those of a study in Suphan Buri province, Thailand. In that study, most of the respondents had inter-mediate knowledge level, positive attitude and satisfactory practices towards avian influenza protection [9].

Our results suggested that our target population was generally aware of avian influenza as a dangerous disease, although they did not clearly recognize some signs and symptoms of severe avian infection. Due to lack of appropriate knowledge, the target population might not respond to externally perceivable symptoms, including diarrhea, severe muscle aches, and difficulty in breathing and convulsion that indicated severe H5N1 virus infection. These findings were similar to a study carried out by Heath Education Division, Department of Health Service, MOPH, Thailand. They found that 69.1% of respondents did not know the symptoms such as high fever, headache, myalgia, sore throat, cough, and pneumonia. Information about signs and symptoms of avian influenza would probably induce rapid responses to improve the prevention practice of avian influenza infection [10].

In this study 73.1% had high level of overall knowledge score. Housewives who had prevention knowledge such as cooking chicken and eggs at enough temperature were 89.2% and washing hands after handling the bird with bare hands 87.7%. It was found that the attitude to preparing

raw poultry and others food on the same cutting board was 53%, nearly half (53%) took care about cleanliness, and 54.5% avoided contacting any live bird. Although most of the housewives had high knowledge scores, nearly half of the housewives had hygienic practice in poultry handling and precautionary behavior towards avian influenza.

Our results showed that there was no statistical significant difference between participants regarding age, family income or occupation. However, knowledge scores were significantly higher among participants with higher education level. The result corresponded with that of a study in which the person's knowledge scores were significantly higher in high education level and not associated with age or sex [8]. This showed the importance of education in determining the level of knowledge. Like other studies, the knowledge of avian influenza is strongly related to prevention practice. A study showed that high knowledge score was significantly associated with good preventive practice score [7].

In one study, a statistically significant relation-ship has been detected between knowledge level and behavior [11]. So, knowledge about avian influenza should be provided effectively to housewives to promote practice of prevention for avian infection among them.

In conclusion, the results of the study suggested that public media played an important role for delivering avian influenza related information in community and education status of housewives was important to attain correct knowledge on avian influenza infection. More effective health education program about avian influenza than at present should be provided through public media apart from television and radio. Moreover, the information gained from the study might be helpful in developing avian influenza prevention and control programs in future.

ACKNOWLEDGEMENTS

The authors would like to express their sincere thanks to Director-General of the Department of Medical Research (LM), for encouragement and also for funding. We are also grateful to all respondents who participated in this study.

REFERENCES

1. Sarah CC *et al*. Avian influenza in poultry: American knowledge, perceptions, and responses. Available from URL: http:// www. food policy in stitute.org
2. World Organization for Animal Health (WOIM). Update on avian influenza in animals (type H5). Available from URL: http://www. oie.int/ downld/
3. World Health Organization. Cumulative Number of Confirmed Human Cases of Avian Influenza A/ (H5N1) Reported to WHO (online fact sheet). Available from URL:http://www. who.int/ csr/ disease/avian influenza/ country/
4. World Health Organization. Avian influenza – situation in Myanmar (online fact sheet). Available from URL:http://www. who.int/ csr/ disease/avian influenza/
5. United States Department of Agriculture, (September 28, 2006). Avian Influenza Low pathogenic H5N1 vs. highly pathogenic H5N1. Available from URL: http:// www. usda.gov/ wps/portal/!ut/p/_s.7_0_A/7_0_1OB?
6. Center for Diseases and Control and Prevention (CDC). (June 03, 2006). Key facts about Avian Influenza (Bird flu) and Avian Influenza A (H5N1) virus. Available from URL: http:// www.cdc.gov/ flu/avian/gen_info/ qa/htm
7. Fatiregun AA & Saani MM: Knowledge, attitudes and compliance of poultry workers with preventive measures for avian influenza in Lagelu, Oyo State, Nigeria. *Jounal of infection in Developing Countries* 2008: 2(2):130-134.
8. Al-Sheri A-S, Abdel-Fattah M & Hifanawy T. Knowledge and concern about avian influenza among secondary school students in Taif, Saudi Arabia. *La Revue de Sante de la Mediterranee*, 2006, Vol.12 (Supplement N 2).
9. Maton T, Butraporn P, Kaewakangwal J & Fungladda W: Avian influenza protection knowledge, awareness, and behaviors in a high-risk population in Suphan Buri province, Thailand. *Southeast Asian Journal of Tropical*

Medicine and Public Health 2007; 38 (3) May: 560-568.

10. Health Education Division. Results of health behavioral surveillance for the prevention of avian influenza: second round data collection, July 24-27, 2004. Nonthaburi, Thailand Health Education Division, Department of Health Service, Ministry of Public Health, 2004 (in Thai).
11. Uzuner A, Ünalan Cöbek P, Alibaº H, Kýlýç Ý, Sarý M, *et. al*, Doctor's knowledge level and attitudes concerning avian influenza. *Marmara Medical Journal* 2008; 21(2):118-126.

The Myanmar Health Sciences Research Journal, Vol. 22, No. 1, 2010

Service factors related to defaulting TB treatment in Myanmar

**Tin Mi Mi Khaing, *Thin Thin Yee,*Tin Maung Swe,*Myat Myat Moe,*
***Saw Saw,*Si Thu Aung,*Win Maung & ***Aye Htun*

*National Tuberculosis Programme
Department of Health
**Department of Medical Research (Lower Myanmar)
***JICA (MIDC Project)

This qualitative study was conducted in collaboration with Department of Medical Research (Lower Myanmar), JICA (MIDC Project) and Myanmar National Tuberculosis Program in four townships in Myanmar. It aimed to describe factors for defaulting TB treatment among new pulmonary TB patients. In-depth Interviews (IDIs) were conducted with 11 defaulted TB patients. Eighteen Key Informant Interviews (KIIs) were conducted with Township Medical Officers, TB Coordinators, Midwives and General Practitioners. Majority of the IDI respondents received health education prior to their anti TB treatment by health staff. Although health staff explained key messages of tuberculosis, the provision of information and health education was probably hindered by shortage of health man power, increased work load and difficulty in communication with different ethnic groups, pointed out by key informants. Time spent for doing health education session and content of the session were different and it depended on type of TB patient. A few IDI respondents were not traced and sought by health personnel. Majority of DOT providers were family members but many defaulted TB patients took the drugs by themselves. DOT supervisor was the key person responsible for patient regularly taking drugs, allowing adequate time spent to discuss with family members and checking the blister. It is essential to ensure frequent supervision on DOT provider in order to improve their capacity. The findings highlighted that ensuring effective, complete pretreatment health education for every TB patients with reference to BHS guideline, conducting initial home visit for every TB patients, motivating BHS for effective DOT, enhancing early missed dose tracing and practicing repeated health education throughout the treatment course were essential for reducing defaulter rates and more effective TB control.

INTRODUCTION

The rapid increase in the incidence of tuberculosis (TB) in developing countries and its re-emergence in the developed world create World Health Organization (WHO) to declare TB as a Global Emergency in 1993. One third of the world population is infected with TB (WHO, 1999). Eight million of new cases every year and nearly 3 millions TB deaths occurred in the world [1]. More than 90% of TB cases are living in the developing world highly related with poverty. Despite effective chemotherapy and cost-effective TB control strategy are available, TB control activities are facing the combined threats of HIV epidemic and multi-drug resistance problems.

Myanmar is still one of the 22 high burden countries throughout the world and its position was 19th in 2005 [2]. In Myanmar, TB is still a public health problem. It was third priority disease and is an increasing trend. Non-adherence of TB treatment leads to various dangerous consequences like

multi-drug resistant TB (MDR-TB) to both patient and the community. Myanmar has adopted DOTS strategy since 1997. However, it achieved 70% case detection rate and 85% treatment success rate in 2006. The very long duration of endeavour and performance was observed to obtain those two targets. It is important to sustain and maintain the achievement of 70/85 which mainly depends on intensive case finding and case holding activities. A lot of efforts and activities were implemented to obtain high treatment success rate. Although national data was satisfactory, some townships and states and divisions did not get 85% treatment success rate.

In Myanmar, most of the townships achieved the targets, treatment success rate 85% and defaulter rate less than 5%. Defaulter rate of a few townships are more than 5% namely Maubin (9%), Thanatpin (8.1%), Yaksauk (19%) and Amarapura (8.4%) in 2005 cohort. Defaulting TB treatment will not only reduce cure rate and treatment success rate of National TB Programme but also become high chance of transmission to community and development of multi drug resistant-TB and death. Previous studies on defaulters were related to patient's factors [3, 4, 5].

Thus, it was essential to point out the reasons for defaulting anti-TB treatment leading to get the possible interventions for increasing in treatment success rate of the National TB Programme. The findings from this study will be valuable and useful to National TB Programme to maintain the already achieved targets of 70/85. It was believed that the findings from this study will be input for further improvement in case holding and to reduce the defaulter rate.

Objective

- To describe service factors for defaulting TB treatment among new pulmonary TB patients in Maubin, Thanatpin, Yaksauk and Amarapura townships in 2006.

MATERIALS AND METHODS

This study was conducted in collaboration with Department of Medical Research (L M) and JICA (MIDC Project) and Myanmar National Tuberculosis Program. The study was conducted in four townships in Myanmar.

Study design

The cross-sectional descriptive study using qualitative approach was conducted.

Study period

It was conducted from September 2007 to September 2008.

Study areas

According to 2006 National TB Programme annual report, four townships which had high defaulter rate were selected. The study areas were Maubin Township in Ayeyawady Division, Thanatpin Township in Bago Division (East), Yaksauk Township in Shan (South) State and Amarapura Township in Mandalay Division.

Study population

- Tuberculosis patients (both male and female), 15 years of age and above, who defaulted TB treatment

Operational definitions

Defaulter

The patient whose treatment was interrupted for two consecutive months

DOT provider

Anyone who watches the TB patient swallow the anti-TB drugs till the entire course of treatment.

Missed dose tracing

Missed dose tracing was defined as the retrieval system of TB patient who had not taken anti TB drugs at least one single dose.

- Township Medical Officer (TMO), TB coordinator, Basic Health Staff (BHS) who had the experience for defaulting TB treatment.

- General Practitioner (GP) who had the experience in defaulting TB treatment in each study area.

Sample size

For Key Informant Interview, there were a total of 18 participants, 4 from Yaksauk Township, 4 from Thanatpin Township, 5 from Maubin Township and 5 from Amarapura Township. For In-depth Interview, there were a total of 11 patients, 4 from Yaksauk Township, 2 from Thanatpin Township, 2 from Maubin Township and 3 from Amarapura Township.

Data collection

In-depth Interviews were conducted with 11 defaulted TB patients and 18 Key Informant Interviews were conducted with TMOs, TB coordinators, midwives and GPs. After the consent was obtained from participant, interviewers recorded the conversation by cassette recorder and some were also recorded by note takers.

Data analysis

All interviews were recorded, sorted, transcribed and listened repeatedly. Quality control check was performed in the field for completeness and if any error was detected, research team members checked with cassette recording. Matrix was used for data display. Qualitative data analysis was done manually. After data analysis, round table discussion with Programme Manager (TB), researchers from DMR(LM) and investigators.

Ethical consideration

During data collection, names of persons included in the study were not mentioned. Face-to-face interview, recording and photographing were requested prior to conduct interview.

RESULTS

Background information of defaulters

There were 7 males and 4 females participated in in-depth interview. Age of those patients ranged from 15 years to 70 years. Majority of the patients were married and from low socioeconomic status including labourer.

Factors related to defaulting

Possible reasons for defaulting were categorized into two - factors related to providers (service factors) and factors related to patients (patients' factor)

Service factors

Pre-treatment health education

Duration of TB health education session varied from 10 minutes to 45 minutes (average was 15 minutes). Half of patients could mention the health education provided by health staff. By triangulation of both KII and IDI revealed that most of TB patients got TB information from health staff.

"I was suffering from TB. When I went to clinic they (TB staff) explained that not to stay closely with family, not to sleep together with children, to take drugs regularly, to ingest drugs in the early morning. Treatment may take 6 months for first time and 8 months for second time. Sputum should be examined after 2 months. Whenever I went, they talked for at least 30 minutes"

(31-year-old male agricultural worker)

"Pre-treatment health education was given to patient who had been diagnosed as TB with sputum results at the time of registration. Firstly, I told them what TB was. It was not hereditary disease. Their wrong beliefs and mode of transmission were explained. After that next step was about the drugs concerned with TB and regular taking of treatment should be systematic. If they do not take the drugs, drug resistance will occur. Treatment will be difficult. Those were the main things I told. Talking to one patient would last for 15 minutes. Some asked questions so that I repeated again. Some only listened to me but did not ask. I told to all new patients, 5-6 patients per day. It was high case load. When there was low case load, I gave

health education to 2-3 patients for one day. Sometimes, when two patients came together, I told those two patients together ".

(public staff)

Majority of key informants did not mention the checking questions after the completion of health education session. But one key informant carried out checking question:

"I take health education session for 1 hour. After telling the facts, I repeat the sentence and ask the questions whether they absorb or not. If they forget, I tell them to look at the Tumbling Kettle (Pyit Taing Dong) Book[*]*."*

(public staff)

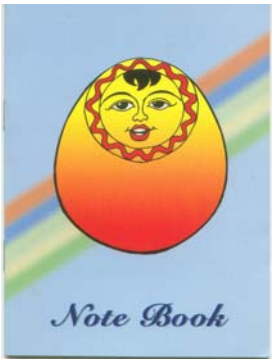


Fig.1. Tumbling Kettle (Pyit Taing Dong) Book*

Almost all key informants discussed about duration of treatment (6 months, 8 months), the importance of regular treatment, drug resistance and the consequences of inter-rupted treatment. Many key informants discussed about the time of drug administration and side effects of drugs, follow-up sputum examination and prevention of TB. A few key informants told patients about the cause of TB and mode of transmission and contact tracing. Only few emphasized on anti-TB drugs are free of charge and TB is curable. None of interviewee of IDI mentioned that TB is curable. Moreover, health staff did not discuss about the drug arrangement for those patients travelling to another township or transfer out. Only one IDI respondent mentioned about the DOT provider.

"Sayama told me like that: Have the breakfast 2 hours after taking that drug. Do the exercise in the morning. Don't take the medicine yourself. DOT has to be done by your spouse. Don't take the drugs by your hand. Your wife will do it. Take the drug with water. The drugs have to be taken daily till 6 months without interruption. If you interrupt, you have to start from the beginning"

(46-year-old male weaver)

Missed dose tracing

Some key informants did not experience with the term missed dose. General Practitioners were not familiar with the missed dose tracing activity. Many key informants mentioned that the missed dose activity was done together with other occasions such as through messenger who lived near the defaulted patient, during home delivery, during home visit to TB patient and during supervision for other purposes. However, some of the key informants conducted this activity.

"Missed dose can be detected only when patient did not come to TB Center and collect the drugs. Midwife understood missed dose tracing. They had training and they did initial home visit. But missed dose tracing was weak. I (TB staff) had no facility and shortage of man power, no junior TB worker, only 4 staff working with 2 vacancies."

(public staff)

A few defaulted patients were not traced and sought by health personnel. The majority of patients had been visited and enquired about their conditions at least once by health personnel.

Drug distribution

The drug distribution system was different from one township to another township. One township is using Stop TB Kits for

[*]The Tumbling Kettle (Pyit Taing Dong) Book is Treatment Record Book for TB patient which also includes health education message. TB patient can continue taking treatment at any public TB centre if he/she can show this book.

category (1) regimen. Therefore, kits were delivered and decentralized. However, the remaining 3 townships that are using 4 Fixed Dose Combinations were practicing different system of delivering of drugs either from TB center or hospital to patients themselves or family DOT providers or DOT supervisors. Sometimes, chance messenger (lui-kyone) collected and brought the drugs for nearby patients with the use of Tumbling Kettle Book (named Pyit Taing Dong).

"I am happy if drugs were regularly obtained. My real residence was Pantanaw. But it is very close to Maubin so that I collect drugs from Maubin. Drugs will be provided whoever came to collect the drugs if this Tumbling Kettle Book *was in hand."*

(31-year-old female agricultural worker)

All patients willingly answered that they got anti-TB drugs free of charge.

"I did not buy the drugs. I did not pay the money for drugs. Because Sayama said that there is no need to pay when I asked her. Those drugs were provided by government."

(55-year-old male agricultural worker)

DOT provider and supervisor

Majority of DOT providers were family members but many defaulted TB patients took the drugs by themselves. Some key informants told that the distance between patient's residence and midwife's residence would determine the different types of DOT provider. Although some key informants were afraid to assign NGO members as DOT providers because of their competency in side-effect management and their attitude, a few key informants said that NGOs might be helpful once they were trained properly.

"MCWA members collect the drugs from me and I disburse one month supply to them. They know the day when the drug was run out. When the patient lives in their ward, they collect the drugs by themselves and they bring and call the patient. I trained them. I give pamphlets to them."

(public staff)

Majority of key informants mentioned that DOT supervisors could not emphasize TB as a whole because a variety of projects were performed at the level of sub-center midwife. The most common problem for supervision was the over work load of health staff.

"They (BHS) have many projects. I (TB staff) always request. When a lot of midwives were chattering each other (sound like "Pi-kyi" "Pi-kyi") I told them very hardly. Among their projects, I requested them very hardly to note and to write even one single fact of TB."

(public staff)

For quality supervision, provision of transport facility and actual traveling perdiem should be motivating factors for DOT supervisors. So, they did not conduct supervision routinely and only practiced in ad-hoc basis.

Patient factors

Most common reasons for defaulting TB treatment as mentioned by defaulters were getting adverse side effects of anti-TB drugs, relief of symptoms, move to other township without informing to respective health staff, little understanding of patient about TB and TB treatment and stopping of TB treatment by specialists.

DISCUSSION

Majority of the patients were received health education prior to their anti-TB treatment by health staff. Although health staff explained key messages of tuberculosis, the provision of information and health education was probably hindered by shortage of health man power, work load and difficulty in communication to different ethnic groups as pointed out by key informants. Time spent for doing health education session and content of the session were different depend on type of TB patient (new and follow-up).

Missed dose was defined as the drugs which cannot be taken by TB patient. Missed dose

tracing was defined as the retrieval system of TB patient who had not taken anti-TB drugs at least one single dose. The missed dose tracing was probably weak because it is not routinely practiced in the township TB Programme and shortage of man power. Missed dose can not be known timely because it was related to interval of drug issue. Regards to drug distribution system, the use of Tumbling Kettle Book was essential for patients. If anyone came to collect the drugs with this book, there would be no difficulty in availability of drugs.

Health staff assigned trained active MCWA member as DOT provider in very few number. To organize and to provide training on DOT providers were the activities to be improved for future planning. DOT supervisor was the key person responsible for patient's taking drug regularly, allowing adequate time spent for discussing with family members and checking the blister. It is essential to ensure frequent supervision on DOT provider to improve their capacity.

The most important type of TB supervision will be initial home visit to TB patients which is the main activity and back bone to eliminate the defaulters. Thus, if the support can be arranged only for initial home visit, it can motivate DOT supervisor. Moreover, the motivating factor other than supplies and support might be recognition of their performance which was necessary to sustain the good performance of DOT provider and supervisor. Provision of health information to patients by specialist was the area to be strengthened because they were the most reliable person in dealing with TB patients. The pre-treatment health education was the important among many factors for defaulting TB treatment because it was related with other factors for defaulting TB treatment such as adverse side effects, sense of well being and travel without informing to respective health staff.

Recommendation

In the light of operational research findings and round table discussion with Project Manager of NTP facilitated by researchers from Department of Medical Research (Lower Myanmar), the following recommendations were drawn.

- To ensure effective, complete pre-treatment health education for every TB patients with reference to BHS guideline
- To practice repeated health education throughout the treatment course
- To conduct initial home visit for every TB patients
- To enhance early missed dose tracing
- To promote effective utilization of IEC
- To increase staff motivation for effective DOT
- To conduct township Continuing Medical Education Session (CME) regularly including TB chapter
- To advocate health care provider (specialist) for NTP guideline
- To strengthen collaboration and co-ordination with NGOs for TB Control activities
- To introduce pretreatment counseling

ACKNOWLEDGEMENT

We would like to appreciate Dr. Than Tun Sein, Director, Socio-Medical Research, Department of Medical Research (Lower Myanmar) for necessary guidance and support to successfully complete the study and also to researchers (Dr. Le Le Win, and Dr. Myo Myo Mon) for sharing their experience and active participation in qualitative research training, data processing and data analysis processes. In addition, we would like to extend our gratitude to the all participants, Township Medical Officers, General Practitioners, Tuberculosis team leaders and Basic Health Staff from the study townships who spent their time and for their contribution and cooperation. The funding for the study was provided by MIDC project (JICA).

REFERENCES

1. World Health Organization. The International Union Against Tuberculosis and Lung Diseases and the royal Netherlands Tuberculosis Association. Revised international definitions in tuberculosis control. *International Journal of Tuberculosis and Lung Diseases* 2001; 5: 213-215.
2. WHO. Global TB control, surveillance, planning and financing, 2007.
3. Hla Naing & Myint Aung. Review of the social factors influencing the defauter cases among tuberculosis paients in Kyaingetone Tuberculosis Center, Myanmar. *Myanmar Medical Journal* 1996 June; 41(2): 22-29.
4. Thuzar Han, Kyi Kyi Thinn, Win Maung & Aye Htun. Time factor and reasons related to tuberculosis treatment defaulting in Yangon (2002). *Myanmar Medical Journal* 2005; 49(1-4):19-24.
5. Kyaw Khaing. Factors responsible for defaulting treatment among the new smear positive pulmonary tuberculosis Zone (1) TB Treatment Center (1999) Dissertation, MMedsSc (Public Health) Yangon, Institute of Medicine (1): 2000.

The Myanmar Health Sciences Research Journal, Vol. 22, No. 1, 2010

Immunohistochemical study of human papillomavirus infection in squamous cell carcinoma of oral cavity

**Thar Htet San , *Yi Yi Myint , *Wai Wai Khaing , *Sann Sanda Khin , *Phyu Synn Oo, *Myo Htet Htet Khaing,, **Khin Thi Lwin & *Htun Naing Oo*

*Department of Medical Research (Central Myanmar)
**Department of Pathology, University of Medicine (Mandalay)

Human papillomavirus (HPV) is a human DNA oncogenic virus. It has been firmly established as a causative factor for cancer of the cervix. However, the extent of involvement of human papillomavirus in causation of some other cancers is still controversial. Oral cancer is included as one of such cancers. This study was carried out to investigate the involvement of HPV E6 oncoprotein in oral cancer cases in Myanmar by immunohistochemistry in 40 cases. The primary antibody used in this study was CHEMICON International Mouse Anti-Human Papillomavirus 16, 18 E6 (C1P5) monoclonal antibody. The HPV 16/18 E6 oncoprotein was detected in 2 out of 40 (5%) oral cancer cases. The immunopositive cases included one squamous cell carcinoma case of tongue and that of gingiva. Findings from this study revealed that although the involvement of human papillomavirus infection existed in oral cancers in Myanmar, its magnitude may be low and not significant.

INTRODUCTION

Human papillomaviruses (HPV) are small double-stranded DNA viruses and are responsible for pathological conditions ranging from benign skin warts to invasive cervical carcinomas [1]. This human DNA oncogenic virus has been proved to be implicated in the carcinogenesis of several cancer particularly squamous cell carcinoma (SCC) of the cervix and anogenital region [2]. Almost all cervical cancer cases are related to HPV infection. Clues of HPV infection were also detected in a variety of other cancers such as vulvar squamous cell carcinoma, oropharyngeal squamous cell carcinoma, oesophageal squamous cell carcinoma and anal squamous cell carcinoma [3, 4, 5]. However, these cancers have stronger nature of multiple etiology and so sole responsibility of HPV as causative oncogenic agent could not be demonstrated as in cervical cancer.

HPV composes of several serotypes, the prevalence of which vary among different geographical regions. High-risk HPV types are predominantly associated with malignancy of various organs. Two of the high-risk HPV types, type 16 and 18, are responsible for as much as 70% of cervical cancer cases. And one study showed that infection with high-risk HPV types 16, 18 and 33 has been detected in a high percentage of patients with several types of cancers including anal cancers [6]. Geographical variation in the distribution of HPV types in association with cervical cancer has been studied well. One study group investigated specimens of cervical cancer patients from 22 countries and reported that HPV 16 was the predominant type in all countries except Indonesia [7].

In Myanmar, two studies showed that HPV 16 is the predominant genotype associated with cervical cancer; presence of HPV 16 DNA in 80% of all HPV-DNA positive cervical cancer cases using PCR-RFLP technique [8] and in 64% (27/42) of invasive cervical cancer cases using non-

isotopic in-situ hybridization method [9]. However, little is known about the impact and pattern of involvement of HPV in squamous cell carcinoma of other organs in different geographic regions.

The recent invented HPV vaccines (eg. Gardasil) intend to be used as effective primary prevention against cervical cancer by preventing infection with HPV types 16 and 18. The impact of these targeted genotypes in a variety of other cancers needs to be investigated so that we can predict the potential benefit of HPV vaccination in addition to reducing cervical cancer mortality and morbidity. To fulfill this knowledge gap, this study was carried out, as a starting pace, to provide the data of our country regarding impact of HPV types 16 and 18, which are preventable genotypes by vaccine, in oral squamous cell carcinomas.

Oral cancer is one of the human cancers which are increasing in number in the world. In Myanmar, according to data from Yangon Cancer Registry, occurrence of oral cancer including lip, tongue, major salivary gland, gum, floor and other parts of the mouth and unspecified part of mouth and pharynx, is 5.91% of total patients for 27 years period (1974-2000) and it is fourth commonest cause of cancer morbidity and mortality [10].

It is generally accepted that long-term tobacco, alcohol and betel quid consumption are the major environmental risk factors for the development of oral squamous cell carcinoma [11,12]. However, some studies noted that the incidence of a subset of oral cancers, including tongue and tonsillar cancer, was increasing in patients who had no exposure to these traditional risk factors or with an exposure time not long enough for malignant transformation [12, 13].

MATERIALS AND METHODS

Tissue samples

A total of 40 oral cancer tissue specimens were collected from Mandalay General Hospital and University of Dental Medicine (Mandalay). The mean age of these 40 patients (33 males, 7 females) was 50.98 years, within the range of 27 - 71years.

Histopathology examination

For histopathology examination, the tissue samples were routinely fixed in 10% formalin, passed through dehydration and clearing and embedded in paraffin. Sections were cut at 4 μm thickness and stained with haematoxylin and eosin. Histopathology examination was done on each case by two pathologists independently and evaluated the histopathologic parameters. If there was discordant finding, both pathologists examined together using multi-headed microscope till final agreement was obtained.

Immunohistochemistry

The most representative tumor tissue block was chosen from each case and 3-4 μm sections were taken to poly-L lysine coated slides for immunohistochemical staining. CHEMICON International Mouse Anti-Human Papillomavirus 16, 18 E6 mono-clonal antibody (Clone C1P5) (1:100 dilution) was used as primary antibody. Autoclaving at 121°C for 20 minutes using 1 mmol/L EDTA buffer pH 9.0 was used as antigen retrieval.

The immunoperoxidase staining procedure was performed at room temperature by using Dako LSAB+ (Dako K0690). LSAB+ included two steps. First step was application of swine anti-mouse antibody conjugated with biotin. The second was application of strepavidin-biotin-peroxidase complex. Final colour development of the sections was carried out with 3,3'-diamino-benzidine. Cervical cancer tissue sections previously known to react with primary antibody were used as positive control. For negative control, TBS was replaced for primary antibody. Immunohistochemical staining was graded as negative (no staining) and positive (immunoreactive brownish staining) for HPV 16 and 18 E6 protein expression.

Evaluation

A case with more than 10% immunopositive tumour cells was regarded as an immunopositive case. At least 200 tumour cells were evaluated for every case.

Statistical analysis

Statistical analysis was performed with statistical software (SPSS 13.0). Expression of HPV 16 and 18 E6 protein was statistically tested against clinicopathological characteristics.

RESULTS

Clinicopathological features

Clinicopathological features of the patients are shown in Table 1. In our study, there was male predominance. Majority of study cases were betel chewers and/or smokers. Well differentiated SCC cases were also predominant (85% of total cases). Site distribution of study cases is as in Table 2.

Table 1. Clinicopathological features

	Number of patients (%)
Age (years)	
Range	27-71
Mean age	50.98
Sex	
Male	33 (82.5)
Female	7 (17.5)
Tobacco smoking	
Present	22 (55)
Absent	18 (45)
Betel chewing	
Present	21 (52.5)
Absent	19 (47.5)
Histologic grade	
Well-differentiated	34 (85)
Moderately differentiated	5 (12.5)
Poorly-differentiated	1 (2.5)

Table 2. Site of 40 oral cancer cases with HPVE6 positivity

	Number of patients	
	HPV E6(-)	HPV E6(+)
Buccal mucosa	20	-
Tongue	5	1
Gingiva	11	1
Floor of mouth	2	-

Immunohistochemical analysis

Figure 1 (A-D) shows the microphotograph of haematoxylin and eosin (H&E), and immunohistochemical analysis of HPV E6.

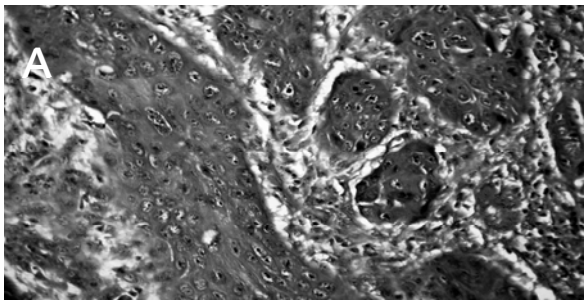
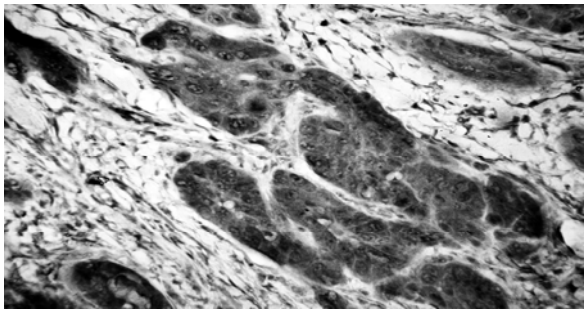
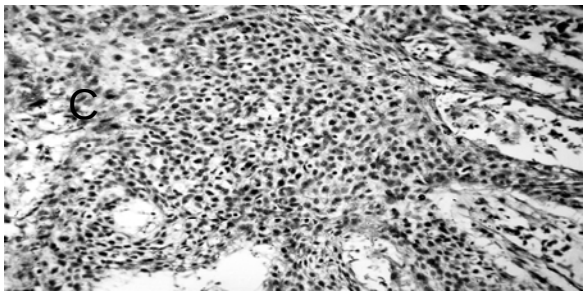


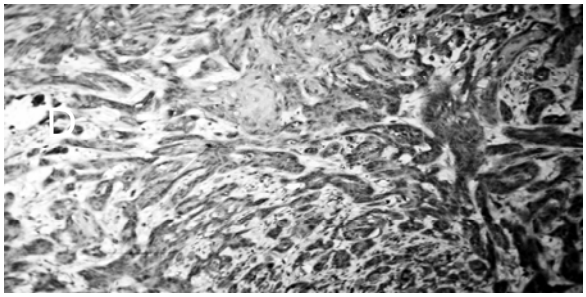
Fig.1(A). SCC of gingiva of 62-year- old male patient (H & E, 400X)



(B). Same case, HPV E6 positive (Immunoperoxidase, 400X magnification)



(C). Positive control of IHC staining (known case of HPV 16 positive cervical cancer case) (Immunoperoxidase, 200X magnification)



(D). Negative control, omitting of primary antibody (Immunoperoxidase, 200X magnification)

HPV 16 and 18 E6 immunopositivity was present in 2 cases (5%) out of 40 cases. HPV 16 and 18 E6 immunoreactivity was mainly seen in the cytoplasm of tumour cells with occasional nuclear staining (Fig. 1B). Immunoexpression of HPV 16 and 18 E6 protein was not significantly associated with age (P=0.508), sex (P=0.323), tumour site (P=0.281) and cell differentiation (P=0.281).

DISCUSSION

In our study, we searched for the presence of Human Papillomavirus Type 16 and 18 E6 protein in oral cancer cases by immuno-histochemistry. In previous literature, the prevalence of the HPV genome was reported inconsistently. A study detected high-risk HPV DNA in 3.9% of the biopsy specimens of the oral cavity [14], whereas a large population-based review study reported that the high-risk HPV prevalence in SCC of the oral cavity was 23.5% [15]. In our study, we detected HPV Immuno-positivity in 2 out of 40 cases (5%). It means that HPV may be involved but is not a major problem in causation of oral cancer in Myanmar.

In our study, HPV 16 and 18 E6 immuno-reactivity was mainly seen in the cytoplasm of tumour cells with occasional nuclear staining. The same finding was reported in previous two studies [16,17]. Our study agrees with these two studies in that HPV oncoprotein can be distributed throughout the cell permitting them to interact with a wider range of cellular proteins.

In our study, HPV immunopositive cases were one case each of SCC of gingiva and tongue. So, this finding that one case out of six SCC cases of tongue being HPV immunopositive (Table 2), is concordant with previous studies which showed that HPV is most prevalent in SCC of tongue and tonsils among oropharngeal cancers.

It has been well documented in cervical cancer that the episomal viral DNA frequently integrates into the host genome as HPV-infected lesions progress to cervical cancer. During viral DNA integration, only E6 and E7 genes remained in the host genome [20]. Therefore, the presence of E6 or E7 genes in tumour tissues may better represent the real HPV infection. So, our finding of HPV E6 oncoprotein rather than L1 protein gives more specific evidence that HPV involved in oral carcinogenesis. In our study, one of two HPV immuno-positive cases had no history of betel chewing and smoking showing the possibility that HPV involve in carcinogenesis of oral cancer cases with no history of smoking and betel chewing. Similar finding was also presented in one previous study [12]. We need to further investigate that interesting clue with a more comprehensive study.

Conclusion

According to the findings from this study, we concluded that although the involvement of human papillomavirus infection existed in oral cancers in Myanmar, its magnitude may be low and not so significant. More comprehensive study with larger sample size is recommended to build up more information in regard with HPV involvement in oral cancer carcinogenesis.

ACKNOWLEDGEMENT

The investigators would like to appreciate to World Health Organization as this work is supported by WHO 2008-2009 grant MMR SO3 RB08 RER1 OWER1 OSER1 P6 A10. Our gratitude also goes to senior consultant surgeons, Dr Nu Nu Yi and Dr Htay Htay Yee for their kind help during collection of study cases.

REFERENCES

1. Lowy DR & Howley PM. *Papillomaviruses.* In: *Field's Virology.* 4th edn. Knipe DM, Howley PM, Griffin DE *et al.* (eds), Lippincott Williams & Wilkins. Philadelphia. 2001; 2231-2264.
2. Lancaster WD. Viral role in cervical and liver cancer. *Cancer* 1993; 70: 1794.
3. Zaki SR, Judd R, Coffield LM, *et al*. Human papillomavirus infection and anal carcinoma.

Retrospective analysis by in situ hybridization and the polymerase chain reaction. *American Journal of Pathology* 1992; 140(6): 1345-1355.

4. Morshed K, Korobowicz E, Szymanski M, Skomra D & Goazbek W. Immunohistochemical demonstration of multiple HPV types in laryngeal squamous cell carcinoma *Europian Archive of Otorhinolaryngology* 2005; 262: 917–920.
5. Yao PF, Li GC, Li J, *et al*. Evidence of human papilloma virus infection and its epidemiology in esophageal squamous cell carcinoma. *World Journal of Gastroenterology* 2006; 12(9): 1352-1355.
6. Mao-De L, Min-Jie L, Jian-Er Y & Pei-Hui C. Anal cancer in Chinese: human papillomavirus infection and altered expression. *World Journal of Gastroenterology* 1998; 4(4): 298-302.
7. Bosch FX, Manos MM, Munoz N, *et al*. Prevalence of human papilloma virus in cervical cancer: a worldwide perspective. *Journal of the National Cancer Institute* 1995; 87(11): 796-802.
8. Mu Mu Shwe, Harano T, Okada S, *et al*. Genotyping of the Human Papilloma Virus (HPV-DNA) from formalin fixed paraffin embedded cervical cancer tissues using PCR-RFLP technique (Restriction fragment length polymorphism). 6th Myanmar Pathologist Conference. 2010; Yangon.
9. Moe Moe Aung, Aye Aye Than, Ne Win, Khine San Yin & Aye Aye Myint. Association of Human Papilloma Virus Type 16 with invasive carcinoma cervix. 6th Myanmar Pathologist Conference. 2010; Yangon.
10. Soe Aung. "Cancer Statistics Year 2000 (Yangon, Mandalay, Taunggyi) (With Summary Data For the Preceding Years).Yangon Cancer Registry, Dept. of Radiotherapy and Medical Oncology, Yangon General Hospital." 2000; Yangon.
11. Tsantoulis PK, Kastrinakis NG, Tourvas AD, Laskaris G & Gorgoulis VG. Advances in the biology of oral cancer. *Oral Oncology* 2007; 43: 523-534.
12. Li G & Sturgis EM. The role of human papillomavirus in squamous carcinoma of the head and neck. *Current Oncology Reports* 2006; 8:130-139.
13. Golas SM. Trends in palatine tonsillar cancer incidence and mortality rates in the United States. *Community Dentistry and Oral Epidemiology* 2007; 35: 98–108.
14. Herrero R, Castellsague X, Pawlita M *et al*. Human papillomavirus and oral cancer: the International Agency for Research on Cancer multicenter study. *Journal of the National Cancer Institute* 2003; 95: 1772- 1783.
15. Kreimer AR, Clifford GM, Boyle P & Franceschi S. Human papillomavirus types in head and neck squamous cell carcinomas worldwide: a systematic review. *Cancer Epidemiology, Biomarkers & Prevention* 2005; 14: 467-475.
16. Guccione E, Massimi P, Bernat A & Banks L. Comparative analysis of the intracellular location of the high- and low-risk human papillomavirus oncoproteins. *Virology* 2002; 293 (1): 20-25.
17. Füle T, Máthé M, Suba Z, *et al*. The presence of human papillomavirus 16 in neural structures and vascular endothelial cells. *Virology* 2006; 348: 289-296.
18. Samir K *et al*. Human papillomavirus (HPV)-related oropharyngeal nonkeratinizing squamous cell carcinoma: Characterization of a distinct phenotype. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology & Endodontics* 2006; 101: 339-45.
19. Gillison ML, Koch WM, Capone RB *et al*. Evidence for a causal association between human papilloma-virus and a subset of head and neck cancers. *Journal of the National Cancer Institute* 2000; 92: 709-720.
20. Zur Hausen H. Papillomaviruses and cancer: from basic studies to clinical application. *Nature Reviews Cancer* 2002; 2: 342–350.

The Myanmar Health Sciences Research Journal, Vol. 22, No. 1, 2010

Prevalence of hypertension and its associated risk factors in Pintaya Township, Southern Shan State

Khin Myat Tun, Han Win, Khin Thet Wai, Theingi Thwin, Zaw Myint, Tin Oo, Moh Moh Win, Moe Thida Kyaw & Maung Maung Myint

Department of Medical Research (Lower Myanmar)

Hypertension is an important cause of cardiovascular mortality and morbidity. This study was conducted in Pintaya Township, Southern Shan State in April 2009 to determine the prevalence of hypertension in adults (18-59 years) and identify associated factors of hypertension focusing on lifestyle, smoking and alcohol drinking, food and beverage consumption patterns in the study population. It was a community-based cross-sectional study inclusive of nested case-control component. Both quantitative and qualitative methods of data collection were used. A total of 431 subjects, 221 from urban and 210 from rural areas were recruited. The overall prevalence of hypertension and prehypertension were 47.1% and 26.5%, respectively, according to the JNC-7 criteria. Hypertension prevalence in urban area was 47.5% and 46.6% in rural area. Regarding the significant risk factors; increasing age ($p<0.001$), history of hypertension ($p<0.001$), and stroke ($p=0.01$) in first degree relatives, body mass index ≥25 ($p=0.001$), waist-hip ratio ≥0.8 in women ($p=0.002$), serum creatinine >1.3 mg% ($p=0.03$) were significantly associated with hypertension. Qualitative data supplemented existing misconceptions related to hypertension, opinions on monosodium glutamate use and high blood pressure and perceived information needs. In conclusion, there was a potential to develop cardiovascular complications indicated by high level of prehypertension and hypertension in this study. Thus, effective public health education and urgent precautions are needed, such as life style modifications including well-balanced diet and increased physical activity.

INTRODUCTION

Hypertension is the common cardiovascular disorder and it is an important public health problem of global dimension, because its disease burden is getting higher in both developed and developing countries [1].

The prevalence of hypertension varies widely among different populations. Based on the cut-off value of hypertension (≥140 mmHg for systolic blood pressure and ≥90 mmHg for diastolic blood pressure, regardless of the age), its prevalence ranges from 5.2% to 70.7% among adults in many parts of the world [2]. Given this high disease burden of cardiovascular disease, hypertension becomes a more important health priority because it is a major risk factor for many cardiovascular diseases, especially stroke and ischaemic heart disease. About two-thirds of stroke and one-half of ischaemic heart disease were attributable to high blood pressure globally [3].

Although hypertension is a premier risk factor for cardiovascular diseases (CVD), which is easily recognized if sought, and can be treated effectively, less number of people with hypertension are aware of their hypertension status and much less number of them are taking regular treatment. Moreover, control of hypertension among them is quite low [4]. Treatment of high blood pressure (BP) has been consistently reported to reduce the risk of CVD [5]. Consequently, the prevention of risk

factors for CVD is a public health priority worldwide [1].

According to a previous cardiovascular diseases survey, the prevalence of hypertension was 12.4% for rural areas and 14.5% for urban areas in Myanmar [6]. A large-scale community-based survey carried out in selected townships in lower and upper Myanmar in 2004 showed that the overall prevalence of hypertension among people aged 18 years and above was 21.3% [7].

The results of this study may assist in finding out ways and means for prevention and proper management of hypertension in the district.

Objective

The aim of the study is to determine the prevalence of hypertension and to reveal the associated factors in the adult population (18-59 years) of Pintaya Township in Southern Shan State.

MATERIAL AND METHODS

Study design

A cross-sectional community-based prevalence study and a nested case-control study from the screened population were conducted.

Study area and population

The study area were both urban and rural setting of Pintaya Township. A total of 431 subjects, 221 from urban and 210 from rural areas, were included in the study. For the nested case-control study, 50 cases and 105 controls were recruited.

Inclusion criteria

1. Adults of working age group between 18 to 59 years
2. Both sexes
3. Those who live in the study area
4. Those who have given an informed consent

Exclusion criteria

1. Those who are critically ill
2. Pregnant and lactating mothers
3. Migratory workers (temporary residents)

Sample size and sampling procedure

Assuming that the prevalence of hypertension in working age group (18-59 years) in Pindaya Township being 50% conservative estimate, marginal error of 0.05 and 95% confidence interval for two sided comparison, the required sample size was 384≈400. The team was able to recruit 162 men only and more women (269) summing up to 431.

For the nested case-control study, the sample size of 50 in hypertension group and 105 in those without hypertension (1:2) achieved 80% power to detect a difference in odds of risk factors between the group proportions of – 0.2105 (effect size) for one sided Z test with pooled variance at 95% confidence level. To ascertain risk factors, 50 cases with hypertension according to the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 7 classification) and 2 controls for each case matched by age group (under 40 years and ≥40 years) summing up to 105 were selected consecutively.

Methods of data collection

Both quantitative and qualitative methods of data collection were used. Face-to-face interviews were conducted by trained interviewers by using pre-tested questionnaire. Specific anthropometric measurements and biochemical tests observing standard procedures (for cases and controls) were done following an informed consent.

Questionnaire included demographic characteristics, lifestyle, personnel habits such as smoking and alcohol drinking, food and beverage consumption, and co-morbidity. Blood samples were taken for biochemical tests such as total and HDL cholesterol, random blood glucose and serum creatinine.

For qualitative data, two mixed Focus Group Discussions (FGD) in Pindaya (urban) were joined by 8 males and

8 females; and two mixed FGDs in rural area were joined by 9 males and 9 females. Altogether 13 in-depth interviews were conducted (6 in urban area and 7 in rural area) including 5 males and 8 females with high blood pressure and additional three key informants.

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured after the subject had rested for 15 min, using a standardized aneroid sphygmomanometer by well-trained personnel. BP was taken in a lying position. Measurements were taken twice with 10 minute interval and the mean was taken for all cases. Participants were advised to avoid cigarette smoking, alcohol, caffeinated beverages and exercises for at least 30 min before their BP measurement. The Korotkoff phase I (appearance) and phase V (disappearance) were recorded for the SBP and DBP, respectively.

Medical officers gave proper treatment for hypertensive patient if he/she was newly diagnosed. And if the patient was treated with antihypertensive medication, the doctors reviewed and made necessary adjustments.

Working definitions

Cut-off points for BP measurement in this study used the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC-7) [8].

Classification of blood pressure (BP)			
Category	SBP mmHg		DBP mmHg
Normal	<120	and	<80
Prehypertension	120-139	or	80-89
Hypertension, Stage 1	140-159	or	90-99
Hypertension, Stage 2	≥160	or	≥100

Data management and data analysis

Data entry was made by EPI DATA software and analyzed by SPSS version 16.0. Conversion tables were used to calibrate self reported dietary intake of salt and monosodium glutamate into comparable international units in grams. For qualitative data, themes and subthemes were analyzed.

RESULTS

Background characteristics

In this study, the mean age of men in urban area was slightly higher than women (43.4±11.6 vs. 38.5±11.2 years) whereas the reverse was true in rural area (38.5±11.2 vs. 42.9±10.5 years). Education level of men in both urban and rural area was higher than women, ie.35.8% of men (58/162) were educated up to high school level and above compared to 26.4% of women (71/269). Seventy-two percent of men (116/162) and 68.8% of women (185/269) in both sites combined were currently married. Nearly 90% of men (144/162) and 75% of women (201/269) were dependents.

Prevalence of prehypertension and hypertension

Prevalence of prehypertension, stage 1 and stage 2 hypertension were 26.5%, 29.2% and 17.9%, respectively, and overall prevalence of hypertension was 47.1%. Men were less likely to have hypertension than women (42% vs. 50.2%, p=0.09). The prevalence of hypertension in urban and rural areas were similar (47.5% vs. 46.6%, p=0.86). Prevalence of hypertension increased steadily with age; 26.2 % in 18-24 years, 33.4% in 25-39 years, 54.6% in 40-54 years, and 67.4% in ≥55 years (p<0.001) (Figure 1).

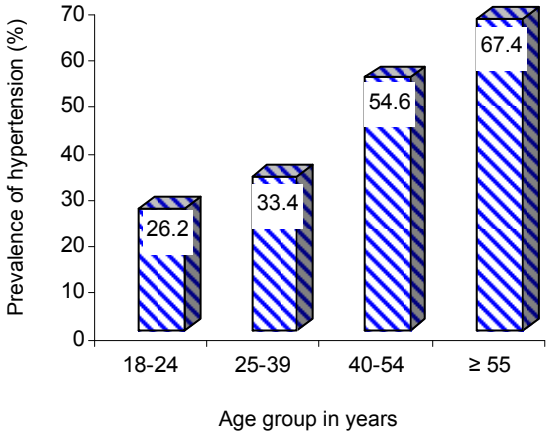


Fig. 1 Prevalence of hypertension in different age groups

Hypertension and associated risk factors

Education, occupation, marital status and household members were not found to be associated with hypertension in this study (Table 1).

Table 1. Crude odds ratio and 95% confidence intervals for demographic characteristics, family history and personal habits of cases and controls

Characteristic	Case (n=50)		Control (n=105)		Crude OR	95% CI	p value
	No.	%	No.	%			
Demographic characteristics							
Education							
Never	3	9.7	9	11.7	1.0		
Monastic	28	90.3	68	88.3	1.2	0.3-4.9	0.8
Primary, Middle High, Grad	19	86.4	28	75.7	2.0	0.5-8.5	0.3
Marital status							
Never & ever married	17	34.0	33	31.4	1.0		
Current married	33	66.0	72	68.6	0.9	0.4-1.8	0.8
Occupation							
Dependent	4	8.3	14	14.7	1.0		
Employed	44	91.7	81	85.3	1.9	0.6-6.1	0.3
Odd jobs	2	33.3	10	41.7	0.7	0.1-4.6	0.7
Household members							
<5	23	46.0	47	44.8	1.0		
≥5	27	54.0	58	55.2	0.9	0.5-1.9	0.9
Family history							
Hypertension in first degree relative							
No	19	38.0	71	67.6	1.0		
Yes	31	62.0	34	32.4	3.4	1.7-6.9	0.0005
Stroke in first degree relative							
No	36	72.0	93	88.6	1.0		
Yes	14	28.0	12	11.4	3.0	1.3-7.1	0.01
Personal habits							
Drinking							
Never	40	80.0	76	72.4	1.0		
Ever (current & past)	10	20.0	29	27.6	0.7	0.3-1.5	0.3
Smoking							
Never	38	76.0	65	62.5	1.0		
Ever (current & past)	12	24.0	39	37.5	0.5	0.2-1.1	0.09

History of hypertension (OR=3.4, 95% CI=1.7-6.9) and stroke (OR=3, 95% CI=1.3-7.1) in first degree relatives had a significant association with hypertension (Table 1), but not with personal history of stroke and diabetes mellitus. Both smoking and alcohol drinking habits were not related to hypertension (Table 1). Similarly, daily salt intake, monosodium glutamate (MSG) consumption and daily oil consumption were not found to be significant risk factors for hypertension (Table 2).

Table 2. Crude odds ratio and 95% confidence intervals for dietary intake, obesity indexes and biochemical parameters of cases and controls

Characteristic	Case (n=50)		Control (n=105)		Crude OR	95% CI	P value
	No.	%	No.	%			
Dietary intake							
Daily salt intake per person							
<5 gram	9	18	25	23.8	1		
≥5 gram	41	82	80	76.2	1.4	0.6-3.3	0.41
Daily MSG intake per person							
<0.6 gram	11	22	25	23.8	1		
≥0.6 gram	39	78	80	76.2	1.1	0.5-2.5	0.80
Daily oil consumption per person							
<32 gram	33	66	83	79	1		
≥32 gram	17	34	22	21	1.7	0.8-3.7	0.14
Obesity indexes							
Body mass index (BMI)							
<25	33	66	92	87.6	1		
≥25	17	34	13	12.4	3.6	1.6-8.3	0.001
Waist-hip Ratio							
<0.8	4	15	26	47.3	1		
≥0.8	23	85	29	52.7	5.2	1.6-6.9	0.002
Biochemical parameters							
Total cholesterol							
≤200 mg%	35	70	80	76.2	1		
>200 mg%	15	30	25	23.8	1.4	0.7-2.9	0.41
HDL cholesterol							
>35 mg%	47	94	88	83.8	1		
≤35 mg%	3	6	17	16.2	0.3	1-1.2	0.08
Serum creatinine							
≤1.3mg%	41	82	98	93.3	1		
>1.3mg%	9	18	7	6.7	3.1	1.1-8.8	0.03

Body mass index (BMI) ≥25 (OR=3.6, 95% CI=1.6-8.3) and waist-hip ratio ≥0.8 in women (OR=5.2, 95% CI=1.6-16.9) were also associated with hypertension (Table 2). Regarding the biochemical parameters, serum creatinine >1.3 mg% had an association with HT (OR=3.1, 95% CI=1.1-8.8). But total and HDL cholesterol were not found to be associated with HT (Table 2).

Qualitative findings

Perceived causes of hypertension included: drinking liquor, salty food, oily food, facing with fearful situation, under mental stress, aggressiveness, unhappiness, family history of stroke and high blood pressure, consuming pork and high amounts of MSG, some kind of food such as bitter gourd, corrianda leaves, fermented bamboo shoots, canned fish, lack of physical exercise and exposure to cool air etc. Additional opinions and attitudes on MSG use were provided during in-depth interviews:

Some answers of subjects on consumption of monosodium glutamate included the followings:

"We've heard that MSG can cause high blood pressure but we can't avoid eating MSG"

"I can't eat anything without MSG. It's tasteless".

"We can't afford to use meat/ dried fish for seasoning. We can only use MSG at cheaper price for improved taste of soup and salads".

Tests of drinking water samples

Five drinking water samples from rural and urban areas of Pintaya Township were tested in the laboratory of Department of Medical Research (Lower Myanmar). pH, total dissolved solid, total alkalinity, total chloride and sodium were analyzed (Table 3).

pH of all water samples were within the acceptable range (6.5-8.5). But total dissolved solid from sample A of Kyauksu Village was 1600 ppm which was unacceptably high. Total alkalinity and total chloride were normal in all samples. Sodium content of the two samples from Pywe Hla Village and one from Pone Ta Loke Pond were 28, 42 and 32.8 ppm, respectively, and were unacceptably high. Acceptable ranges of total dissolved solid, total alkalinity, total chloride and sodium are shown in Table 3.

Table. 3. Results of the tests on drinking water samples from urban and rural areas of Pintaya Township

Water sample	pH	Total dissolved solid (ppm)	Total alka-linity (ppm)	Total chloride (ppm)	Sodium (ppm)
Sample A (Kyauksu Village)	6.7	900	168	14	9.6
Sample B (Kyauksu Village)	6.7	1600	80	18	11.6
Pone Ta Loke Pond	6.7	300	92	50	32.8
Sample A (Pywe Hla Village)	6.7	200	240	43	28
Sample B (Pywe Hla Village)	7.1	200	192	64	42

Acceptable pH= 6.5-8.5
Total dissolve solid (WHO References)
Excellent= less than 300 ppm
Good= between 300& 600 ppm
Fair= between 600& 900 ppm
Poor= between 900&1200 ppm
Unacceptable= >1200 ppm
Acceptable chloride content=less than 250 ppm
Acceptable sodium content=less than 20 ppm

DISCUSSION

Hypertension is a common health problem in developing countries including Myanmar. Its prevalence is currently rising steadily and varies widely among different populations which are somewhat dependent on factors such as race, lifestyle and degree of urbanization as well as by region [9, 10].

Using JNC-7 guidelines in the present study, the prevalence of hypertension was found to be 47.1%. The combined prevalence of both prehypertension and hypertension was very high (73.6%). Moreover, the prevalence of hypertension was also high in younger age groups; 26.2% in 18-24 years and 33.4% in 25-39 years.

There were no previous studies in this particular area comparable to this study using JNC-7 guidelines. However, by using the conventional BP measurements and cut-off points, the prevalence of hypertension in rural area of Thonegwa Township in 1998 was 38.4% and it was 15.5% in Pardagyi Village of Kyauktan Township [11, 12]. In 2001, hypertension prevalence in Kanaung and Bonlon Villages of Kyauktan Township

was 21.9% in 15 years and above population [13]. It was 22.4% in Tayokehla and Myaingalay villages of Kayin State in 2002 [14]. According to WHO Stepwise Survey in 2003 conducted in 15 Townships of Yangon Division, the prevalence was 23.5% in 4616 study subjects of 20 years and above (personal communication). The regional variations were clearly observed.

In this study, age was strongly associated with hypertension which was consistent with other studies [10, 15, 16]. History of hypertension or stroke in first degree relatives was found to be the risk factor for hypertension. It may be due to same dietary habits and living conditions among family members rather than racial and genetic factors.

In the present study, hypertension was strongly associated with body mass index (BMI) ≥25 and waist-hip ratio (WHR) ≥0.8 in women. High BMI and high WHR reflect obesity and truncal obesity, respectively. Studies in Ethiopia, Indonesia and Vietnam found that obesity was a principal risk factor for the development of hypertension [17]. Overweight or obesity was significantly associated with high BP [18]. In our study, 34% of cases had BMI ≥25 and 85.2% of cases among women had high WHR ≥0.8. Underlying causes of obesity needed further exploration. Educational efforts on obesity and its risks should be made and established balanced nutrition and increased physical activity. We found that the serum creatinine >1.3 mg% had an association with hypertension. Serum creatinine was an indicator of renal function in human body. Increased serum creatinine may be the cause or effect of hypertension in this study. Prevalence of smoking in 18 years and above population was only 24% and the association between smoking and hypertension was not found.

Most of the study population consumed vegetable oil which contained polyunsaturated fatty acids which was discussed in FGDs and observations. They were believed to lower the blood cholesterol. Thus, blood cholesterol levels were not raised in the study subjects and both total and HDL cholesterol levels were not found to be associated with hypertension.

In this study, the effects of dietary salt and monosodium glutamate on hypertension were not obvious unlike other studies. This may be due to measurement bias in self reported data [19]. Actually, according to the qualitative estimates and observations, people in the locality widely used monosodium glutamate together with salt during food preparations especially soup. Major reasons were lack of availability of other types of seasoning such as fish, prawn, and meat, attracted by palatable taste and easy availability and as a habit. Misperceptions on causes of high blood pressure indicated the requirement for accurate, comprehensive and timely IEC message through locally appropriate media channels.

The sodium content of drinking water from Pywe Hla Village and Pone Ta Loke Pond from urban area was higher than the acceptable level. In fact, the sodium content of the drinking water was only 1% of daily sodium consumption from all food. But when coupled with high daily consumption of salt and MSG, it may contribute to the high prevalence of hypertension and prehypertension in the study area.

Conclusion

There was a potential to develop cardiovascular complications indicated by high level of prehypertension and hypertension in this study. If there is no fully implemented program to prevent and control hypertension, health care costs may increase owing to expected increase in morbidity and mortality. Moreover, the prevalence of hypertension in younger age groups was also high. Health personnel needed to promote regular blood pressure measurement of young age groups <40 years. To control prehypertension and hypertension, effective public health education and urgent precautions are needed in the study area. There is a requirement to emphasize on life

style modifications such as combination in increased physical activity, weight reduction and well-balanced diet.

ACKNOWLEDGEMENT

We like to express our sincere thanks to the Director-General, DMR (Lower Myanmar) for allowing us to conduct the study and also for the funding. We are also grateful to the Township Medical Officer and Staff of Pintaya Township for their kind help throughout the study. Last but not the least, we thank all the study subjects.

REFERENCES

1. WHO, Hypertension. *The World Health Report,* 1997; 39-45.
2. Kearney PM, Whelton M, Reynolds K *et al.* Worldwide prevalence of hypertension: a systematic review. *Journal of Hypertension* 2004; 22:11-19.
3. Lawes CMM, Vander Hoorn S, Law MR, Elliott P, MacMahon S & Rodgers A. Blood pressure and the global burden of disease 2000. Part 2: estimates of attributable burden. *Journal of Hypertension* 2006; 24: 423-430.
4. Chobanian AV, Akris GL, Black HR, Lee WC *et al.* The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. *JAMA* 2003; 289: 2560-2572.
5. Staessen JA *et al.* Cardiovascular protection and blood pressure reduction: a meta analysis. *Lancet* 2001:1305-1315.
6. Ministry of Health, National Health Plan, 1996-2000.
7. Khin May San, Tracy Sein, Myo Oo, Myint Soe Win, *et al.* NCD Risk Factors in Myanmar population. In: *Scientific Programme & Abstracts of 12th Medical Specialities Conference of Myanmar Medical Association*, Yangon, 18– 25 Nov. 2005.
8. Joint National Committee on the Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. The Seventh Report of the Joint National Committee on the Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report. *JAMA* 2003; 289: 2560- 72.
9. Altun B, Arici M, Nergizoglu G *et al*. For the Turkish Society of hypertension and Renal Disease. Prevalence, awareness, treatment and control of hypertension in Turkey (the Paten T study) in 2003. *Journal of Hypertension* 2005; 23: 1817-23.
10. Kearney PM, Whelton M, Reynolds K *et al.* Worldwide prevalence of hypertension: a systematic review. *Journal of Hypertension* 2004; 22:11-19.
11. Tin Nu Swe, Than Pe, Tin Zaw, Khin Saw, Khin Thet Wai, Phyu Phyu Aung *et al.* An investigation of hypertension in Thonegwa Township. *Myanmar Health Research Congress, Programme and Abstracts* 1998: 90.
12. Han Win, Khin Myat Tun, Maung Maung Toe, San Hla Mu *et al.* Prevalence of hypertension in Pardagyi Village, Kyauktan Township. *Myanmar Health Sciences Research Journal* 2005;17(1):22-26.
13. Survey report on prevalence of hypertension in Kyauktan Township. Department of Medical Research (Lower Myanmar), 2001.
14. San Shwe, Ohnmar, Kyu Kyu Than, Than Tun Sein, Aung Thu, *et al.* Prevalence of hypertension in two selected villages of Kayin State, Myanmar. *Regional Health Forum* 2004; 8: 104- 112.
15. Sonmez HM, Basak O, Camci C *et al*. The epidemiology of elevated blood pressure as an estimate for hypertension in Aydin, Turley. *Journal of Human Hypertension* 1999; 13:399-404.
16. Aytekin NT, Pala K, Irgil E, *et a*l. Distribution of blood pressure in Gemlik District, Northwest Turkey. *Health Society Care Community* 2002; 10: 394-401.
17. Kunz I, Schorr U, Klaus S, *et al*. Resting metabolic rate and substrate use in obesity hypertension. *Hypertension* 2003; 36: 26-32.
18. Erem C, Yildiz R, Kavgaci H *et al*. Prevalence of diabetes, obesity and hypertension in a Turkish population (Trabzon City). *Diabetes Research & Clinical Practice* 2001; 54: 203-8.
19. Cohen HW, Hailpern SM, Fang J, Alderman MH. Sodium intake and mortality in the NHANES// follow-up study. *American Journal of Medicine* 2006; 119: 275. e7-275. e14.

The Myanmar Health Sciences Research Journal, Vol. 22, No. 1, 2010

Social burden of malaria on family in Hlegu Township, malaria endemic area

**Le Le Win, **San Shwe, ***Than Win, *Thandar Min &*San San Aye*

*Health Systems Research Division
**Department of Medical Research (Lower Myanmar)
***Malaria Control Programme, Department of Health

To explore social burden of malaria on family in a malaria endemic area, a cross-sectional study was conducted in Hlegu Township during March 2009. After getting consent, 56 family members who had suffered from malaria involved in eight sessions of Focus Group Discussions and pile sorting, four each for males and females. About half had suffered from malaria at least once in their life. While most males were working in the forest, most females were either working odd jobs or dependants. Social burdens were explored in sequence, like health, financial issue, family, social, children and personal factor. They all agreed that the poorer the people, the more they were affected badly from the malaria, particularly those who worked in the forest. The family suffered many hardships when major earner and the housewife got the disease. Of them, the housewife was the key person who had to handle all of the household matters - finding household expenses, treating the patient, for children's schooling, paying back the loan, and trying to get more loans. The findings revealed that human misbehaviour, malaria-related environment and poor knowledge about the disease largely influenced for having these burdens.

INTRODUCTION

Malaria is the second priority disease in Myanmar and about 72% of the population live in malaria endemic areas [1]. Myanmar contributed to the highest number of reported deaths in SEAR (about 65%) [2].

Despite the implementation of malaria control activities in Myanmar, problems are still existing - high morbidity and mortality, and other problems in anti-malaria activities. These include spreading of multi-drug resistance, uncontrolled population movement possibly leading to epidemic prone situation, indiscriminate use of anti-malaria medicines and rising trend of mortality in forested and hilly areas due to poor accessibility to health services, which are mostly contributed by the human behavior which, in turn, affects health promoting and preventing activities of malaria. This indicates that there is an interrelationship between malaria and human behavioural factors that result in social burden of malaria [2, 3, 4].

Social burden was defined as cost of society imposed by the existence of disease such as decisions related to reproduction, education and economic matters, which are the influences of culture, beliefs and political context [3, 5]. Since the perceptions of social burden vary among individuals, families, societies and cultures, we need to know what social burden of malaria means to local people. Then we can plan locally appropriate and acceptable strategies to reduce morbidity and mortality of malaria. Till now, little is known about social burden of malaria and we thus attempt to conduct this study to fulfill this gap.

Objectives

The objective is to explore social burden of malaria on family in a selected malaria endemic area.

MATERIAL AND METHODS

The study was conducted in two rural health centres (RHC) of Hlegu Township, malaria endemic township of Yangon Division during March 2009. The northern part of the study area is close to Bago Yoma mountain range and surrounded by dense forest reserve and Nga-moe-yate Dam. It takes at least over night from the nearest villages to the forest fringe by bullock cart and it is the only available transport for the majority of the residents. Inside the forest, the roads are not accessible for the cars and there is only narrow footpath for the people. From 2006 to 2008, in the study area, malaria was the leading cause of morbidity when compared to other diseases. Similarly, mortality from malaria is on the top of the list [6].

Ethical approval was obtained from The Ethical Review Committee of Department of Medical Research (Lower Myanmar). A cross-sectional exploratory study design was applied. After getting consent, Focus Group Discussion (FGD) and Pile sorting were carried out, eight FGDs, four sessions in each RHC (Min-kone-san-pya and Hle-hlaw-inn) as shown in Table 1.

Table 1. Number of study respondents involved in FGD

Sex	Min-kone-san-pya			Hle-hlaw-inn			Total
	FGD I	FGD II	Total	FGD I	FGD II	Total	
Male	8	6	14	8	8	16	30
Female	6	6	12	8	6	14	26
Total	14	12	26	16	14	30	56

Fifty-six family members who were literate, aged 18 years and above, who had similar background and who had experience with malaria illness participated in the study.

RESULTS

Most were males (53.6%). Majority, both sexes, was in the working age group of 25 to 55 years (82.1%), married (82.1%), and attained minimum of middle level education (76.8%). They stated that all the hardships of the family were badly influenced if head of household and the mother, labourers in the forest, were the sufferers. Above all, if the affected person was poor, they were in the worst condition.

Treatment seeking

All respondents said in their villages every house had at least one family member who had malaria because forest-related job is the main job available in the study areas. The majority said they first tried home remedies, which were much cheaper than the western medicine. When the traditional medicine could not comfort them, some used the western medicine. Some took quinine before going into the forest where they could buy it from the road-side shop. However, for the majority, they said an immediate action taken was not possible for all of them, particularly for the western medicine. They explained that majority caught fever while working in the forest, which made them difficult to reach the village for timely treatment, and/or most were unable to pay for the treatment.

Understanding the malaria disease

Almost all the respondents recalled their parents' saying, '**…** *these thick forests [from the study area] were widely spread with malaria and once a person got it, he or she would be ill seriously. No one could stand it especially the people came from the plain area (i.e., non-endemic malaria area). Because their power of resistance was much lower than us and they were more likely to die'*.

However, different understandings about disease were explored among study respondents. Regarding the cause of malaria, majority, mostly the men said that it was due to mosquito-bite and mosquito-breeding sites. Most of both sexes said about unhealthy environment, unhygienic water source, working from morning to midnight in the malarial forest without protection, careless behaviour such as skipping meals, eating at an inopportune time, drinking unclean water and sleeping without bed net.

Different views on malaria were also mentioned that malaria was a kind of treatable and fatal disease, unpredictable disease, disease with pretence and/or *ahpjaou* (old disease of illness).

Social burden

When compared to other diseases, they said they suffered from malaria the most because majority earned as odd-job persons in the forest. About half (44.6%) of the respondents had suffered from malaria at least once in their life. Malaria has caused family sufferings widespread; it brings the family many misfortunes - socially, financially, physically and psychologically.

Due to previous and current attack of malaria, they said poor people, those who work in the forest, those who come from non-endemic area, those who have low immunity, mobile people and those who lack knowledge had more chance to get malaria, and hence affected all related persons. They faced with different types of social burden which, in sequence, were health, financial issue, family, social, children and personal factor. They all agreed that, generally, all the hardships of the family were badly influenced if head of household and the mother were the sufferers. But if the person was poor, his or her family was affected badly. Among all affected persons, they said women were the key persons for handling household matters like finding household expenses, treating the patient, for children's schooling, paying back the loan, and trying to get more loans.

They identified social burden as an individual category, however, they said these happened almost at the same time that could not be distinguished explicitly. The followings illustrate this point.

If a head of household suffered from malaria, family was affected badly. While male respondents did not point out specifically about this issue, most female respondents had an opposite view. Most women said: *"When they (husbands) got disease, we had many hardships. We had money only when they were back from forest. However, we had to use this for their treatment. So we had to find a job and also find for loan that needs to pay interest. Most moneylenders didn't trust us whether we could pay back because he (husband), who is the main earner, was ill. Therefore, sometimes, we could only borrow half of what we wanted."*

If the mother was ill, some school-age children had to stop attending school. Even though they were in debt, the mothers only gave up when they were in the incapable situation. One of them expressed her feeling as:

"My daughter was attending 10th grade when I got malaria. I couldn't tell her to leave the school. But with her own decision, she dropped from school and it was only three months before her final examination. There was no one at home who could help me. I could neither sit for a long time nor do housework. I had three sons and one daughter only. I feel sorry for her. I have a plan to enrol her again next year when I feel better. But she encouraged me not to worry for her that she would find a job."

(a female respondent, 42 years, married, 3rd grade, dependant)

Due to lacking in care of proper treatment, finally a poor person died and left behind the family with the sorrows and troubles. Through their experiences, majority of both sexes said: *"In our community, if the expired person was main earner, the family affected more desperately than other people did. Some family members had to move out to other places individually. There were some children whose mother died of malaria. Separately, the children had to live with the relatives, where they became insecure and mischievous children. Some had to sell their all belongings like gold, house, garland, and had come to the dead end."*

Being poor people with low income, majority said they were caught in a vicious cycle of sufferings from malaria - stop working, facing with various problems,

treating the disease, working again when became well - then the same incident happened on and on.

DISCUSSION

Most people in the study area had suffered from malaria at least once in their life. They believed that mosquito bite and their careless behaviour and unhealthy environment were the main reasons for getting malaria. People from another malaria endemic area of Hlegu Township believed mosquito bite (79.6%), drinking stream water area (36.7%) and eating banana (6.6%) were the causes of malaria [7]. Malaria, the disease of poverty, widely and severely affects the people living in malaria endemic area, especially the life of the poor people. Malaria affected the changes in household behaviour in response to the disease that resulted in broad social cost [3, 8, 9]. Similarly, social losing from malaria was observed among study community, where majority are forest-related daily wage earners. It was mainly influenced by human misbehaviour, malaria-related environment and poor knowledge about the disease.

Though some were knowledgeable about the disease, mainly, the men were working in the forest when getting malaria and resulted in cerebral malaria or died of malaria as the forest area is far away from the nearest health facility and it is not feasible for the health workers to reach to the affected person on time. A study also documented the similar situation and suggested that early diagnosis and treatment at peripheral health services could reduce severe malaria morbidity and mortality [8]. For the worst, majority did not regard malaria seriously. Consequently, they were confronted with sufferings from disease, which could end up with death, financial hard-ship, inconveniences in social dealing as a cyclic process. The findings revealed that human misbehaviour, malaria-related environment and poor knowledge about the disease largely influenced for having these burdens.

ACKNOWLEDGEMENT

We would like to express our gratitude to Director-General, Department of Medical Research (LM) for allowing conducting this study. Thanks are due to Township Medical Officer from Hlegu Township, Station Medical Officer from Phaung Ggyi Station Health Unit and staff from Hlegu Township for organizing respondents and helping in conducting the study. We wish to express our gratitude to WHO for funding this study. Last, but not the least, we are grateful to our study respondents, without them, this study would not be accomplished.

REFERENCES

1. Ministry of Health. National Health Plan (2006 - 2011) Yangon. Ministry of Health. 2006.
2. World Health Organization. *Communicable diseases: malaria*. 2005. Available from URL: http:// www. searo.who.int/
3. World Health Organization. *Report of the scientific working group meeting on malaria.* TDR/SWG/03. Geneva: WHO. 2004.
4. Heggenhougen HK. Social, political, economic and inequity issues related to malaria resurgence and inequalities in access to treatment for and prevention of malaria. In: *Report of the Scientific Working Group Meeting on Malaria*. Geneva: World Health Organization. 2004; 134-138.
5. Jones COH & Williams HA. The social burden of malaria: what are we measuring? *American Journal of Tropical Medicine and Hygiene*. 2004; 71 (2 Suppl) 156-161.
6. Department of Health. Health Profile of Hlegu Township. 2008.
7. Hla Shein, Than Tun Sein, Soe Soe, Tin Aung, Ne Win & Khin Saw Aye. The level of knowledge attitude and practice in relation to malaria in Oo-do village, Myanmar. *Southeast Asian Journal of Tropical Medicine and Public Health* 1998; 29(3); 546-549.
8. Tanner M & Vlassoff C. Treatment seeking behaviour for malaria: a typology based on endemicity and gender. *Social Science and Medicine* 1998; 46 (4-5): 523-532.
9. Khanum S & Singh A. Health, poverty and human security: illustrations from malaria control programmes in the SEA Region. *Regional Health Forum* 2007; 11(1); 33-44.

The Myanmar Health Sciences Research Journal, Vol. 22, No. 1, 2010

Ineffectiveness of a single dose of rifampicin, ofloxacin and minocycline (ROM) chemoprophylaxis in preventing leprosy

**Khin Nwe Oo, *Khin Thet Wai, **Kyaw Myint & **Mg Mg Gyi*

*Department of Medical Research (Lower Myanmar)
**Department of Health

Chemoprophylaxis was carried out on high-risk group of extended contacts of new leprosy cases in Nyaungdon Township, Ayeyawady Division, Myanmar and serological response and new case detection rate were followed for 6 years from September 2003 to August 2009. In October 2003, blood samples were collected from 829 extended contacts after getting informed consent and sera were tested for immunoglobulin M antibodies using Natural Trisaccharide Phenyl-Propionyl–Bovine Serum Albumin (NTP-BSA) ELISA test. From these contacts, 300 seropositives were randomized to chemoprophylaxis as treated and non-treated groups. In each group, 102 were enrolled in adults and 48 in children. A single dose of ROM and RMP (rifampicin) by body weight was administered to treated group of above 15 years, and those below 15 years, respectively. The vitamins were administered to non-treated group. The blood samples of all contacts were collected again in October 2004 and October 2005 and ELISA was carried out on paired samples on one plate. New cases detection was carried out from 2004 to 2009 yearly. The difference of mean OD titers before and after chemoprophylaxis was significantly reduced in treated group compared with non-treated group in adults. The difference of mean OD titers between before and after chemoprophylaxis in treated group was 0.14 and non-treated group was 0.11 and p=0.0004 after 1 year and treated group was 0.08 and non-treated group was 0.01 and p=0.0005 after 2 years. New cases were not detected till 2006 (3 years after chemoprophylaxis). Two, (one each from seronegative and non-treated group), 3 (one each from all 3 groups) and 1 (from seronegative group) contacts developed leprosy in 2007, 2008 and 2009, respectively. One contact developed leprosy from treated group in 2008. However, the numbers of new cases and New Case Detection Rate (NCDR) in treated, non-treated and seronegative groups were not significantly different. Therefore, a single dose of ROM chemoprophylaxis seems to be not effective as presumed within 6 years.

INTRODUCTION

Leprosy elimination was achieved at national level at the end of January, 2003 in Myanmar. With the widespread use of MDT (Multi Drug Therapy), it brings down the prevalence rate (PR) of leprosy. However, until June 2003, New Case Detection Rate (NCDR) was >4/100,000 in some townships of Yangon, Bago, Ayeyawady, Mandalay, Magwe, and Sagaing Divisions and Southern Shan State. NCDR of Ayeyawady was 11.9/100,000 in 2003 [1]. It is because of the long incubation period of the disease or subclinically infected persons and patients' contacts can transmit *Mycobacterium leprae.*

Seroassays can identify subclinical cases and if these could be detected and treated, their infectivity for others would be curtailed and then the incidence of leprosy will be decreased [2]. Chemoprophylaxis or immunoprophylaxis can be considered to prevent the disease in high-risk community. Vaccination with Bacilli Calmette-Guerin (BCG) has shown that it had varying efficacy of 20-80% against leprosy [3]. Use of vaccines has more complex issues

because it needs cold chain storage, using disposable needles and syringes.

In remote areas, chemoprophylaxis is operationally more feasible than vaccination [4]. ROM chemoprophylaxis has a significant role in reduction of antibody titre. A single dose of ROM can kill 96.8-98% of *M. leprae* in mice and 95.75% of bacilli in 9 out of 10 untreated lepromatous patients [5]. There are no serious side effects to combination of ROM therapy to paucibacillary (PB) patients with a single patch.

The bacterial count of a subclinical infection is small, so that, it is unlikely to include a single RMP (rifampicin) resistant mutant. Therefore, the risk of resistance is negligible. If it includes RMP resistant mutant, emergence of RMP resistance is still very unlikely, because a single dose of RMP is insufficient to select resistant mutants [6].

Therefore, we carried out the study of chemoprophylaxis on high-risk group of extended contacts of new leprosy cases in pocket area of our community using ROM.

Objectives

- To compare the difference of mean OD titers before and after chemoprophylaxis
- To assess the role of chemoprophylaxis with the following indicators:
  a) absolute numbers of new cases with adjusted denominator,
  b) New Case Detection Rate (NCDR)
  c) incidence rate (IR) with changes of prevalence rate (PR).

MATERIALS AND METHODS

This study was community-based prospective longitudinal study focusing on changing Optical Density (OD) titers of ELISA results and appearance of new cases after chemoprophylaxis. It was carried out from September 2003 to August 2009. The study area was Nyaungdon Township, Ayeyawady Division. There were 32 new cases including 17 MB cases and 7 MB cases were less than 12 years old children at the end of 2002. The study population was the extended (household and neighbor) contacts of new leprosy cases. Chemoprophylaxis was carried out on high-risk individuals (seropositives) of extended contacts and were chosen randomly as treated and non-treated groups. For chemoprophylaxis, a single dose of ROM or rifampicin was given to cases of above and below 15 years old, respectively, during September 2003. The cases were followed up to 2009 and seroassay was done yearly for 2 years in September 2004 (after one year) and 2005 (after 2 years of chemoprophylaxis).

Extended contacts

The extended contacts comprised households up to neighbor 3. The household contacts were those who lived with a patient. The neighbor 1 contacts were from houses adjacent to both sides of patient's house. The neighbor 2 contacts were from houses adjacent to both sides of neighbor 1 houses. The neighbor 3 contacts were from houses adjacent to both sides of neighbor 2 houses.

Blood sample collection

Two milliliter of blood were taken after getting informed consent from each subject who is over 18 years of age. If the subject is less than 18 years of age informed consent was taken from their parents or guardians. The blood was withdrawn using disposable needles and syringes. The clotted blood samples with cold storage were carried to Department of Medical Research (Lower Myanmar), and Enzyme-Linked Immunosorbent Assay (ELISA) test was carried out between September and October 2003 to get baseline data.

NTP-BSA ELISA test

The ELISA microtitre plates (Fastec microplate U, Fujirebio Inc,) with 96 U-shaped wells were coated with 50µl of 1µg/ml concentration of soluble antigen (NTP-BSA), incubated at 37°C in moist chamber for 2 hours and placed at 4°C overnight.

On the next day, the solution were sucked out and washed 3 times with Phosphate Buffer Saline-Tween 20 (PBST).

One hundred microlitre of blocking agent were added to each well, incubated at 37°C for 1 hour in moist chamber and washed with PBST for 7 times again. Fifty microlitre of test or control sera which was diluted in 1:300 with dilution buffer was added to each well, incubated at 37°C for 1 hour and washed with PBST for 7 times. Fifty microlitre of goat anti human IgM conjugated to horse-radish-peroxidase, diluted in 1:2000 with dilution buffer was added to each well, incubated at 37˚C for one hour and washed with PBST for 7 times. One hundred microlitre of substrate solution (orthophenylene-diamine) was added to each well, incubated in dark box for 15 min and reaction was stopped by stopping solution. OD was measured by ELISA plate reader with wavelength of 492 nm. Mean OD was calculated from duplicate results [7]. Pooled sera from 10 MB and normal apparently healthy subjects of National Blood Bank (NBB), Yangon were used as positive and negative controls, respectively.

Administration of chemoprophylactic drugs

Chemoprophylaxis was carried out during October 2003 after getting ELISA results. Seropositive subjects were given chemoprophylaxis by using single blind, randomized controlled trial. According to sample size determination, the number between treated and non-treated group was the same. Among seropositives (above 15 years old), 2 groups were chosen randomly as treated and non-treated groups and were coded with odd and even numbers. Odd numbers (treated group) were administered chemoprophylaxis using a single dose of ROM (rifampicin 600mg, ofloxacin 400mg and minocycline 100mg) and even numbers (non-treated group) given with vitamins. Colour and shapes of vitamins were same as ROM. The seropositives among children of less than 15 years old were chosen randomly as 2 groups and treated group was given a single dose of RMP by body weight (25mg/kg) and non-treated group given with vitamins.

Follow-up and data analysis

The blood samples were collected again from all extended contacts during October 2004 and 2005 and then ELISA assay was carried out. Each paired samples from October 2003, Oct. 2004 and Oct. 2005 were tested on one plate. OD values of these seropositive contacts of treated/ non-treated groups were calculated and compared. SPSS software version 11.5 was used for data entry and analysis.

Detection of new cases

The suspicious leprosy cases were detected among study community. The suspicious cases are subjects having a single patch with anaesthesia or having one nerve thickening. New cases detection was carried out from 2004 to 2009 yearly.

RESULTS

The difference of mean OD titers before and after chemoprophylaxis was significantly reduced in treated group compared with non-treated group in adult. However, it was not significant in children [8] (Table 1 & 2).

Table 1. The difference of mean OD titers before and after chemoprophylaxis in treated and non-treated groups after one year

Type of contacts	Difference between before and after in groups (mean OD±SD)		P value
	Treated	Non-treated	
Adult (n=102)	0.14±0.09	0.11±0.08	0.004*
Child (n=48)	0.14±0.08	0.12±0.09	0.18 (NS)

*Wilcoxon Signed Rank Test. *p<0.05= significant, NS= not significant

Suspicious cases of leprosy were assessed starting from 2004 (after 1 year of chemoprophylaxis), however, new cases were not detected till 2006 (three years after chemoprophylaxis) among study community (829 extended contacts). In 2007, 2 contacts developed leprosy. Case 1 was household contact of MB case, from non-treated group, and developed leprosy in 2007 as PB case.

Table 2. The difference of mean OD titers before and after chemoprophylaxis in treated and non-treated groups after two years

Type of contacts	Difference between before and after in groups (mean OD±SD)		P value
	Treated	Non-treated	
Adult (n=102)	0.08±0.12 (102 before, 94 after)	0.01±0.08 (102 before, 93 after)	0.0005*
Child (n=48)	0.06±0.12 (48 before, 45 after)	0.02±0.11 (48 before, 46 after)	0.103 (NS)

*Wilcoxon Signed Rank Test. *p<0.05= significant, NS= not significant

Case 2 was neighbor 1 contact of MB case, from seronegative group and developed leprosy in 2007 as MB case. In 2008, 3 contacts developed leprosy. Case 3 was household contact of MB case, from treated group and developed leprosy in 2008 as MB case. Case 4 was household contact of MB case, from non-treated group and developed leprosy in 2008 as MB case. Case 5 was neighbour 1 contact of MB case, from seronegative group and developed leprosy in 2008 as MB case. In 2009, 1 case developed leprosy. Case 6 was neighbour 1 contact of MB case, from seronegative group and developed leprosy in 2009 as MB case.

When NCDR was calculated in treated, non-treated and seronegative groups they were 666.7/ 100,000, 1332.5/ 100,000 and 576.9/ 100,000, respectively. When the differences were analyzed, p values were 0.625, 0.856 and 0.390 among treated vs. non-treated, treated vs. seronegative and non-treated vs. seronegative groups, respectively. The absolute numbers of new cases shown with adjusted denominator were 1/150, 2/150 and 0.87/150 among the treated, non-treated and seronegative groups, respectively. The incidence rate after 6 years was 0.2% per year.

DISCUSSION

The new cases developed in non-treated and seronegative group in 2007 (i.e. after 4 years of chemoprophylaxis). It also developed in treated group in 2008 (i.e. after 5 years of chemoprophylaxis). Therefore, it could be suggestive that chemoprophylaxis seems to prolong the incubation period. All the new cases developed in this study were above 15 years, none in less than 15 years age group. When comparing the absolute numbers of new cases with adjusted denominator, seronegative group has the lowest number among these 3 groups. It is because the other 2 groups were seropositives (that is subclinical infected subjects).

The difference of mean OD titers before and after chemoprophylaxis was significantly reduced in treated group compared with non-treated group in adults. However, NCDR in treated, non-treated and seronegative groups were not significantly different. A single dose of ROM chemoprophylaxis seems to be not effective as presumed within 6 years. Therefore, a single dose of ROM chemoprophylaxis seems to be not effective in preventing leprosy.

ACKNOWLEDGEMENT

This research was funded by International Medical Center of Japan (IMCJ). We would like to thank Dr. Tin Shwe, Laisson Officer, International Leprosy Programme (ILEP), for editing the manuscript.

REFERENCES

1. Ministry of Health, Yangon, Myanmar 2002 In: Leprosy Control Program, Yangon.
2. Ulrich N, Smith PG, Sampson C, Zuniga M, *et al.* IgM antibodies to native phenolic glicolipid-1 in contacts of leprosy patients in Venezuela: Epidemiological observations and a prospective study of the risks of leprosy. *International Journal of Leprosy* 1991: 59; 405 -415.
3. Fine PEM: BCG vaccination against tuberculosis and leprosy *British Medical Journal* 1988: 44;691-703.
4. Smith CM & Smith WC. Chemoprophylaxis is effective in the prevention of leprosy in endemic countries. MILEP2 Study Group Mucosal Immu-nology of Leprosy. *Journal of Infections* 2000: 41; 137-142.
5. Sow JiB, Perani P, Lienhardt C, Diderot V & Grosset J. Bactericidal activity of a single dose

combination of ofloxacin plus minocycline, with or without rifampin, against *M. leprae* in mice and in lepromatous patients. *Antimicrobial Agents and Chemotherapy* 1998: 42; 1115- 1120.

6. Baohong Ji & Grosset JH: Drugs and regimens for preventive therapy against tuberculosis disseminated *Mycobacterium avium* complex infection and leprosy. *International Journal of Leprosy* 1999: 67 (4 Supple); 45-55.
7. Cho SN, Yanagihara DL, Hunter SW *et.al.*, Serological specificity of PGL-1 from *M. leprae* and use in serodiagnosis of leprosy. *Infection and Immunity* 1983: 41; 1077-1083.
8. Khin Nwe Oo, Nwe Nwe Yin, Tin Tin Han, Khin Thet Wai, Kyaw Myint & Mg Mg Gyi. Serological response to chemoprophylaxis in extended contacts in leprosy - a randomized controlled trial. *Japanese Journal of Leprosy* 2008; 77: 3-10.

The Myanmar Health Sciences Research Journal, Vol. 22, No. 1, 2010

SHORT REPORT

Hepatitis B vaccination coverage among trainees in Central Institute of Civil Services (182nd batch, 2009, Phaung Gyi

Min Min Win,Aung Zaw Latt & ***Win Aung*

*Blood Programming Division
**Virology Research Division
***Department of Medical Research (Lower Myanmar)

Hepatitis B virus (HBV) infection is one of the major global health problems. Approximately two billion people have been infected worldwide and more than 350 million are chronic carriers of HBV [1]. HBV infection accounts for 500,000 to 1-2 million deaths each year and is the 10th leading cause of death [2]. The prevalence of HBV infection varies markedly in different geographic areas of the world. In developed countries like Northern Europe and America, HBV infection occurs in <1% of the population. In developing countries of Asia and Africa, HBV infection occurs in 5-10% of the general population [3]. Based on studies conducted in Myanmar during 1981-2000, hepatitis B surface antigen (HBsAg) prevalence among general population is estimated to be 10-12%. This has placed Myanmar in an area of high endemicity [4].

A vaccine against hepatitis B has been available since 1982. Hepatitis B vaccine is 95% effective in preventing HBV infection and its chronic consequences, and is the first vaccine against a major human cancer [1]. In Myanmar, HBV infection is regarded as one of the vaccine preventable diseases taking 8th position among the prioritized list of disease and health condition in National Health Plan (2006-2011) [5]. In Myanmar, plasma-derived HB vaccine has been available since 1997 and a total of 12 million doses have been produced for 13 years. However, most of Myanmar people are not aware to take the HB vaccination even health care workers. This study was conducted with an aim to explore the HB vaccination coverage among the trainees in Central Institute of Civil Services (CICS) (182nd batch, 2009), Phaung Gyi. It was a cross-sectional study and data were collected according to the proforma, and data entry was analyzed manually.

A total of 155 trainees, 43 males and 112 females age ranging between 25 and 45 years participated in this study. Among them, 107 trainees were medical personnel and 48 were non-medical personnel. Among the medical personnel, 5 trainees (1.9%) were not given the HBV vaccine and 2 trainees (4.7%) were given incompletely. Only 100 (93.4%) were given the vaccine completely. Among the non-medical personnel, 39 trainees (81.2%) were not given the vaccine and 8 trainees (16.7%) were given the vaccine completely. Only one trainee (2.1%) was given the vaccine incompletely. Therefore, a total HB vaccination coverage among all trainees was found to be 71.6% (111 out of 155). Among all the vaccinees, 41 (36.9%) were given the DMR plasma-derived HB vaccine and 70 (63.1%) were given other HB vaccines such as Engerix B, Hepavax, Euvax, etc.

It was noted that even among the educated trainees, one-fourth were not given HB vaccination. The proportion of vaccinated persons among the educated trainees of

similar population was much higher when comparing with the findings of similar research in 1998. In this study, only 2.6% of trainees were found vaccinated against hepatitis B. Although the previous study showed that 82% had a perception towards that VHB can be prevented by vaccination, only 71.6% of vaccination coverage in this study finding was still so low even in the officer level [6].

Table 1. Hepatitis B vaccination coverage among trainees in Central Institute of Civil Services (182nd batch, 2009), Phaung Gyi

		Medical personnel n=107	Non-medical personnel n=48
HB vaccination	Complete	100(93.4%)	8(16.7%)
	Incomplete	2(4.7%)	1(2.1%)
No vaccination		5(1.9%)	39(81.2%)

Even among the medical personnel, few have not been given the HB vaccination yet. So non-medical personnel, although they are graduates, have no idea to be given the vaccine. Present study indicates that some people are not aware of prevention of hepatitis B infection although it can be prevented by the vaccine. Since the HB vaccination has been introduced in EPI program in Myanmar in 2003, HB vaccination coverage of newborn baby seems to be satisfactory. However, most of Myanmar adult people have a little knowledge about hepatitis B virus infection and they do not know how to prevent it. So it is necessary to disseminate the knowledge about the hepatitis B virus infection, mode of transmission, the preventable measures including HB vaccination to Myanmar public through the different media widely. This is the preliminary study carried out among the limited sample size within the short period of training course. To obtain the definite situation analysis of HB vaccination coverage of Myanmar people, it needs to be carried out on large population with the community-based survey.

REFERENCES

1. World Health Organization. Hepatitis B, fact sheet number 204. (Revised Oct:2000). Available from URL: http://who.int/inf.fs204 /en/ fact.html.
2. Mahoney FJ. Update on diagnosis, management and prevention of hepatitis B virus infection. *Clinical Microbiology Review* 1999; 12:51-366.
3. Chowdhury A. Epidemiology of Hepatitis B virus infection in India. 2004; 1:17-24.
4. Than Tun Sein, Than Htein Win, San Shwe, Kyu Kyu Than, Ko Ko Zaw and Khin Pyone Kyi. Feasibility of providing hepatitis B birth dose in community setting: a qualitative assessment. *Myanmar Health Sciences Research Journal* 2008; 20(3):133-139.
5. Report of National Health Plan (NHP) (2006-2011). Prioritized list of diseases and health conditions in NHP.
6. Kyaw Oo & Engenie Phyu Aye Thwin. Knowledge, perception and behaviour of Civil Service Officers towards viral hepatitis B infection. *Myanmar Health Research Congress, 2000. Programme and Abstracts,* 34-35.