Genetic and Dental Profiles of Orang Asli of Peninsular Malaysia

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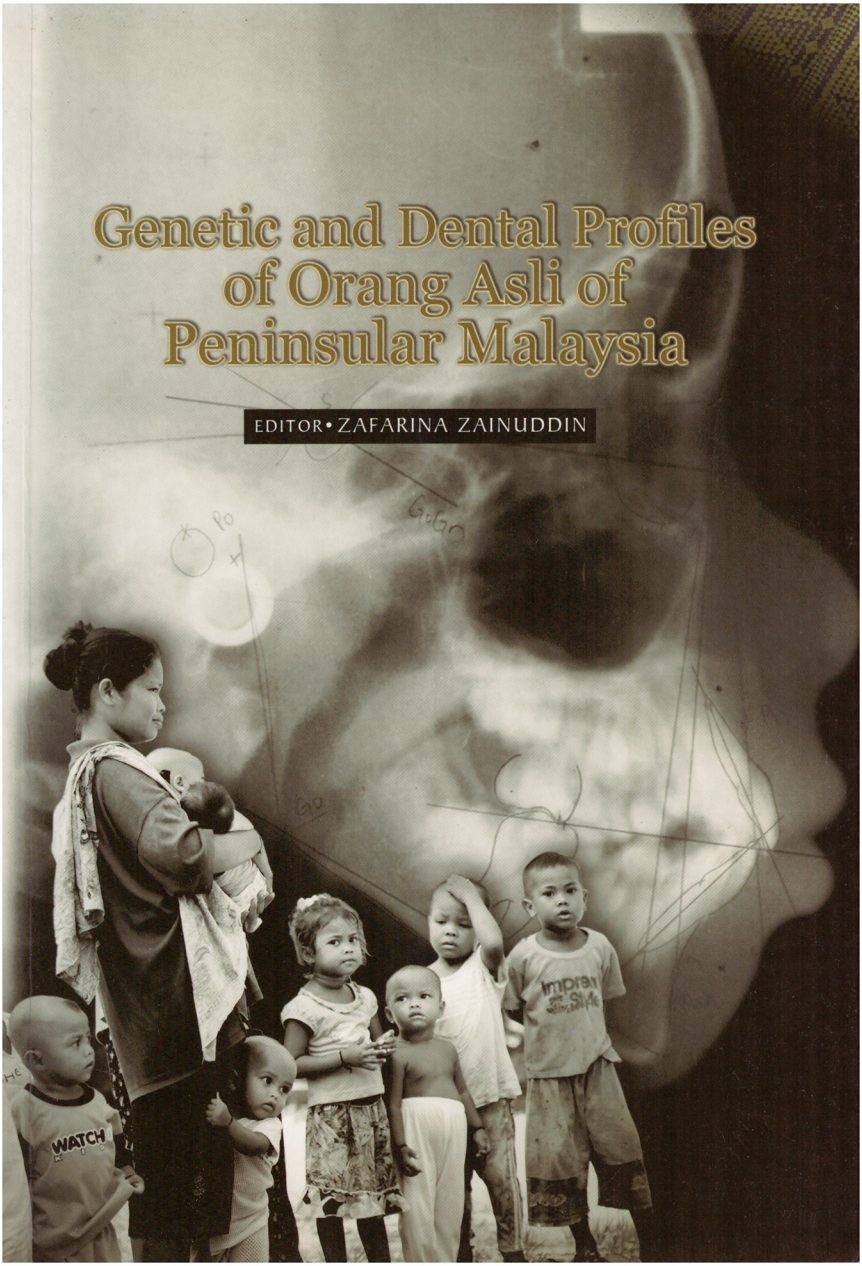

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Contents

Preface

Orang Asli, the earliest inhabitants of Peninsular Malaysia, have been the subject of intense studies since they are believed to be the principal relict population in Southeast Asia. Their relict mitochondrial DNA (mtDNA) has been used to trace the prehistoric migration, along with other archaeological, sociological and linguistic analysis. The Semang, which is one of the Orang Asli tribes in Peninsular Malaysia, were the descendants of the earliest modern human migration to Southeast Asia dated around 40,000 to 65,000 years ago. Studies on the Orang Asli populations, which make up only 0.5% of the total Malaysian population, should provide vital insights into the prehistory of Southeast Asia, especially Peninsular Malaysia.

This book is the first compilation of genetic and dental studies related to the Orang Asli populations. It also includes some information on the historical and social trends of the Orang Asli studies. The genetic studies cover the maternal and paternal DNA markers, and analysis on malaria associated polymorphism and HLA polymorphism. Several biomedical parameters were also studied, along with genetic analysis. The dental studies include the dental identification techniques, occlusal traits analysis and craniofacial morphology of several sub-tribes of the Orang Asli.

There are eight chapters in this book; three chapters each for genetic and dental studies, written by experts in the area. The earlier chapters of this book include some general information on the Orang Asli populations in Peninsular Malaysia. It also incorporates the social and historical trends in the Orang Asli studies, covering the advancement of ethnography, demography, linguistics and other issues pertaining to the Orang Asli studies from 1890 to present. From this review, it is clearly seen that most of the early studies were done by the Western scholars. The British colonial officers and foreign students have also shown large interest in the Orang Asli and had published many articles or theses since then. The local researchers only came into the picture in the last 50 years, which reflect the less number of articles published as compared to the foreign researchers.

The third chapter of this book described the analysis of mtDNA of two Semang subgroups, Jahai and Kensiu, as well as the Modern Malay population. mtDNA is a valuable tool in evolutionary genetics of modern humans and is also an essential means to understand the demographic history of human populations. This chapter reveals that the Modern Malay do not seem to be the direct descendants of the Orang Asli. The Semang showed characteristics belonging to the Australo-Melanesian lineage while the Modern Malay are more closely related to the Austronesian speakers lineage. The small number of haplogroups shared between these populations indicated limited relationship between both ancestors.

In Chapter 4, mtDNA (HVS-1) and Y-chromosome (SRY gene) analysis carried out on Orang Kanaq, which is the smallest Orang Asli subgroup (only 80–90 individuals), showed that this group suffers the ill-effects of genetic inbreeding for decades due to their restriction on intermarriage. Threats from various factors to their survival remain large due to their small genetic pool. This analysis has established that Orang Kanaq is more closely related to Orang Kuala based on their mtDNA polymorphisms while the SRY gene indicated close relationship between all Proto Malay subgroups.

Chapter 5 detailed the biomedical analysis carried on the Jahai, Kensiu, Temuan and Bidayuh (one of the indigenous group of Borneo) and it has indicated that they are now at higher risk for diabetes, hypertension and cardiovascular diseases. These are the consequences from their new lifestyle influenced by urbanization. Their poor dietary habits due to their poor knowledge on good diet have actually contributed to this scenario. Genetic analysis based on HLA marker of these subgroups has revealed that they have a small gene pool, which may represent a population bottleneck or founder effect. Some biomedical test results suggested that malaria infection was present in these groups, eventhough no direct evidence was found. Due to limited health care facilities and poor knowledge in good hygiene practice, they are also at higher risk for parasitic and microbial infections.

The sixth chapter in this book discussed the dental identification techniques used to analyse the diversity of the dental morphological features in few Orang Asli subgroups (Bateq, Jahai, Temiar, Semelai and Temuan). Dental analysis is important since wealth information can be obtained from teeth, one of the last structures to disintegrate, other than bones after death. It can contribute an inordinate body of evidence to the evolutionary anthropologist, the archeologist or the forensic expert to rely on. This chapter describes the dental cast taking procedure, subsequent laboratory routine carried out and how measurements are

made to look into the inter-population dental diversity and relationships between the different groups of Orang Asli of Peninsular Malaysia.

The occlusal traits analysis of the Orang Asli in Chapter 7 has shown that this population is undergoing phases of socio-economic transition. The high prevalence of anterior irregularities and crowding, significant number of posterior open bite and posterior crossbite in adolescence are the response towards changes in their life circumstances. These results contradict the reports of previous analysis, which stated that a nomadic or non-modernized people should display a good anatomical occlusion. This finding indicated that the Orang Asli population is facing an adverse effect from the changes of their lifestyle, which is in accordance with the result of the biomedical analysis.

Besides the dental morphological features, the craniofacial morphology is also discussed in Chapter 8. The craniofacial morphology result is very useful in population affinity study and clinical management since it is population specific. Such study has been carried for the Jahai in order to obtain their craniofacial skeletal morphology measurement, to evaluate the sexual dimorphism in this subgroup by using a multivariate approach and also to compare the results with published data from other populations. These methods have proved that the Jahai, which is one of the Semang, is phenetically different from the northern Mongoloid (represented by the Japanese) and Australian Aborigines. This is in agreement with the mtDNA data which indicated that the Semang is the descendant of Austro-Melanesian lineage, who survived the mongoloidization process by moving into the rainforest and manage to retain their identity till today.

I am indebted to all authors who have contributed chapters in this book and also for their patience in waiting for this book to be published. A special thanks to Professor Tan Sri Dato' Dzulkifli Abdul Razak, the former Vice Chancellor of Universiti Sains Malaysia (USM), who is actually the person that came out with the idea for compilation of studies related to the Orang Asli by local researchers. This book shall be the turning point for developing the Orang Asli research cluster, which is rather important in order to avoid overlapping studies and also to compile precious information of one of the oldest populations in the world.

Million of thanks to Professor Dr. Ab. Rani Samsudin who initiates the first step that makes this book a reality. He is the best mentor one could have. I would also like to thank the Department of Orang Asli Affairs (JHEOA), recently known as JAKOA, for their help and guidance throughout my studies and I believed to other

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Zafarina Zainuddin

CHAPTER 5

Health Status and Genomic Analysis in Four Indigenous Groups in Malaysia

Maude E. Phipps, Timothy A. Jinam, Hong Lih Chun, Umah Rani Kuppusamy, Indran Mathavan and Juli a/l Edo

Introduction

It has been over 70 years since the first report was published in 1931 that provided a glimpse into the health conditions of the Orang Asli. It revealed the presence of endemic malaria, skin disease and various afflictions in the Temiar (Baer, 1999). In the 1970s, Lie Injo and his co-workers at the Institute for Medical Research, Kuala Lumpur, published reports on recounting of various aspects of Orang Asli health. The majority of these reports focused on parasitic disease, nutritional status and several biomarkers linked to genetic polymorphism.

Most studies have identified the Orang Asli as a single homogenous unit. Group-specific studies focused mainly on the Temuan, Temiar, Semai and Semelai, who represent the majority of Orang Asli in west Malaysia. Biomedical studies on the Temuan range from analysis of malarial resistance genes (Baer et al., 1976) to food security and nutritional status in their children (Zalilah & Tham, 2002). In other smaller groups such as the Negrito, data has been scattered and scarce. Reports on high incidences of filariasis in Jahai (Hakim et al., 1995) and goiter in Kensiu (Osman et al., 1995) are examples of studies carried out on Negrito.

There are some reports and publications on health issues affecting the indigenous groups of Borneo, which mainly focused on malnutrition, malaria, filariasis and microbial infections. Some of the earliest biomedical studies on Bidayuh looked at abnormal hemoglobin, G6PD deficiency and hereditary ovalocytosis (Ganesan et al., 1975). More recent publications drew attention to low iodine levels in Bidayuh children (Low et al., 2002) and high rates of nasopharyngeal cancer recorded in the Bidayuh (Devi et al., 2004).

In general, past reports seem to indicate a very different outlook of health in indigenous communities compared to the general Malaysian population. Diseases such as malaria, tuberculosis and filariasis have a higher incidence and prevalence in the indigenous groups, thus giving a sense that these cases are isolated to those groups. However, as Malaysia gears towards socio-economic growth at a rapid pace, changes to the nutrition, lifestyles and environment of minority groups will inevitably occur. As more previously isolated populations become urbanized, health issues affecting them will also change. Diseases such as widespread parasitic infections and malnutrition would become less of a burden, only to be replaced by conditions associated with affluent and sedentary lifestyles such as diabetes and cardiovascular diseases.

Health Indicators

Cardiovascular diseases (CVD) are on the rise and pose major health problems worldwide. Coronary heart disease alone claims more than 7 million lives annually, most of these in developing countries (Mackay & Mensah, 2004). In Malaysia, CVD accounted for 23%–26% of deaths in government hospitals from 1994 to 2001 (Zambahari, 2004). Hypertension, obesity and diabetes have been identified as major risk factors for CVD. Thus, measurement of body mass index (BMI), blood pressure, glucose and cholesterol levels would be helpful indicators of these risk factors.

BMI is frequently used as an indicator of obesity. Obesity is defined as having BMI of more than 30 kg/m^2, but the value itself is not an absolute determinant. Other factors such as waist circumference and body fat percentage should also be taken into consideration.

Blood pressure readings are typically reported as systolic pressure/diastolic pressure, for example, 120/80, which corresponds to pressure when the hearts contracts or is at rest. Hypertension is broadly defined as having blood pressures of over 140/90 mmHg and is attributed to a myriad of factors which includes stress, smoking, high-fat diet and genetic factors. Blood glucose measurement at fasting level typically serves as an indicator of diabetes. Cholesterol is essential for basic physiological functions. However, excess of low-density lipoprotein (LDL) cholesterol can lead to clog blood vessels, resulting in cardio-pulmonary complications. Measurement of total cholesterol includes both LDL and high-density lipoprotein (HDL) levels and serves to estimate the risk of developing CVD.

There is an emerging trend in identifying lifestyle diseases like diabetes, hypertension and CVD with oxidative stress. Oxidative stress occurs when the level of free radicals in the body exceeds that of the antioxidants. Free radicals are strong oxidative molecules which are endogenously produced by the cells during normal cellular metabolisms. Body systems may be also bombarded by loads of free radicals from exogeneous sources such as environmental pollutants, cigarette smoke and radiation. Free radicals have a high propensity to damage and alter other cellular molecules in their 'enthusiasm' to stabilize themselves by abstracting or pairing up with electrons from other stable molecules. LDL oxidation by free radicals has been shown to be the main 'culprit' that initiates the atherosclerotic plaque formation which eventually leads to not only to debilitating and fatal CVDs but also precipitate other serious diseases.

Antioxidants on the other hand are molecules that are able to scavenge or inactivate the free radicals, thus, conferring protection against free radical induced damage. Antioxidants that may be obtained through the diet include beta carotene, vitamins C and E and polyphenols, all from plants. The human body is equipped with an endogeneous antioxidant system that comprises numerous types of metal binding proteins that prevent amplification of free radical formation and antioxidant enzymes that inactivate the free radical molecules. Some of the oxidative indices commonly measured to estimate oxidative stress are malondialdehyde (MDA); a marker for lipid peroxidation, advanced oxidized protein product (AOPP); a marker for damaged proteins and ferric reducing antioxidant power (FRAP). Taken as a whole these indices estimate total non-enzymic antioxidants or reductants. Numerous studies have shown that the oxidative stress levels in Malaysians with diabetes, hypertension and CVD are elevated (Kuppusamy et al., 2005; Indran et al., 2001). As society grows more affluent, the levels of oxidative stress and disease complications increase probably due to reduced physical activity and unhealthy eating habits, exposure to pollutants and other risk factors. Oxidative stress has also been implicated in the etiology of many cancers and Alzheimer's.

In terms of the immunological system, the human major histocompatibility complex (MHC) plays an important role in cell-mediated immune responses due to its involvement in presenting antigenic peptides to antigen specific T-cell receptors. It forms the basis of distinguishing immunologically between self and non-self, hence its importance in protection from or susceptibility to disease and its major role in transplantation. Human leukocyte antigens are coded by HLA genes which display the highest levels of polymorphism in the human genome. More than 2,000 known genetic variants or alleles have been documented (Robinson

et al., 2003) and the number of variants continues to increase as reported on a yearly basis. HLA genes are useful as markers to distinguish populations. Alleles that are common in one ethnic group might be rare or even absent in another. Many previous investigations used older less specific serological methods for testing HLA types and only in recent years have highly specific DNA based techniques been available.

Previous Studies on Health Status of the Indigenous Population

Studies on health parameters in the Malaysian population focus largely on the major ethnic groups. National health surveys on the distribution of BMI, blood glucose, blood total cholesterol and blood pressure were carried out on the Malay, Chinese and Indian ethnic groups (Lim et al., 2000a; Lim et al., 2000b; Lim et al., 2000c; Lim et al., 2000d). Indigenous groups were also included in those studies, but remain anonymous as they were grouped together as 'other indigenous' in previous reports. A study on blood glucose levels in the Orang Asli by Osman et al. (1996) did not to identify the exact subgroups involved. Data on the health status of indigenous peoples of Sarawak is even more meagre. Apart from a study on obesity in which Dayak women were found to be physically less active compared to Malay and Indian women (Ismail et al., 2002), and some reports on tobacco chewing and its consequences, well-documented biomedical data is generally unavailable.

Studies on health parameters such as BMI, blood pressure, blood glucose and total cholesterol in the indigenous groups are lacking. There have been no previous attempts made to assess the oxidative stress levels in these groups. Previously, authors tended to group together the various indigenous communities into generic terms such as 'aboriginal' or 'dayak'. The identification of specific ethnic groups was neglected. The authors are now aware that different lifestyles, environments, socio-economic factors and genetic diversity of the various ethnic groups directly influence their health status.

This study was initiated in order to access the health status and disease risks in some indigenous groups in Malaysia through biomedical analysis, to elucidate genomic diversity and also to gain insight into some of their beliefs and practices in the light of the current, ethical, legal and social concerns. An ethnic specific study is needed as they are in danger of being left out of mainstream research and subsequently miss out on any benefits and knowledge gained from these studies.

Genetic diversity of the Asian population is hitherto under studied despite of their high level of genetic diversity. Current databases document mainly Caucasian or very regional Asian populations. Knowledge of Asia's genetic composition, in particular its' indigenous communities, will create major benefits. The data will allow researchers to determine, with high resolution, the genetic relatedness of Asian populations and the patterns of human migration across Asia.

Biomedical and HLA Analysis

Three Orang Asli subgroups of Peninsular Malaysia (Jahai, Kensiu and Temuan) and one indigenous group of Borneo (Bidayuh) were selected for this study. The Jahai and Kensiu are the subgroups of Negrito, living in west Malaysia. They use dialects of the Austroasiatic language lineage. The Temuan (Proto Malay) and Bidayuh use different dialects of the Austronesian language. A total of 110 Temuan, 107 Jahai, 53 Kensiu and 106 Bidayuh individuals were recruited from six settlements in north and central Peninsular Malaysia and Bau, Sarawak; after University of Malaya Medical Centre (UMMC) ethics committee, official, chieftain and community approvals were obtained. Participants gave both verbal and written informed consent, after they have been explained about the study in the simplest terms using the language that they were most comfortable with.

Basic biomedical parameters such as BMI, blood pressure, blood glucose (non-fasting) and total cholesterol levels were measured. Oxidative biomarkers (lipid peroxidation byproduct) namely MDA and FRAP were also investigated. Blood samples from adults and mouthwash samples from children were collected for further analysis.

The biomedical findings in the studied populations that have been summarized and contrasted with information derived from the general population of the Klang Valley are illustrated in Table 5.1.

Table 5.1 Biomedical indicators in four indigenous groups contrasted with the general population in the Klang Valley

	Temuan	Jahai	Kensiu	Bidayuh	General population in Klang Valley
Age	40.63 ± 13.98	31.36 ± 15.41	31.53 ± 16.82	48.80 ± 14.90	40+
BMI	26.03 ± 0.58*	21.27 ± 3.43	20.95 ± 3.95	24.44 ± 3.80	24
Blood pressure	138/84	140/87	123/79	132/80	120/80
Glucose	5.95 ± 0.36	5.22 ± 0.71	5.08 ± 1.33	6.05 ± 1.63	5.5
MDA	4.36 ± 0.85*	4.29 ± 0.63*	2.18 ± 0.53	2.47 ± 0.72	2.48
FRAP	577.37 ± 106.76	727.45 ± 83.55*	1295.78 ± 86.79*	463.34 ± 102.27	747
Total cholesterol	4.85 ± 0.74*	3.23 ± 1.27	2.90 ± 1.10	4.16 ± 1.20*	4.5

Notes: SPSS Ver 13, Anova *P < 0.05 compared within groups

Body mass index (BMI)

This parameter was only measured from individuals above 18 years of age. Data from children and adolescents were not included as they have different definitions of BMI classification and also those two age groups were under-represented in the Bidayuh and Kensiu. In all four groups, women had higher mean BMI than men. The Temuan had the highest mean BMI, followed by the Bidayuh. Both Jahai and Kensiu have lower mean BMI. The Temuan tended to be generally on the overweight with an average BMI of 26, which is higher than the average BMI of the Klang Valley random population (Lim et al., 2000d). The Jahai and Kensiu are much slender peoples with an approximate BMI of 21.

The percentage distribution of BMI revealed that the Temuan recorded the highest percentage of obese and overweight individuals (57.6%), followed by the Bidayuh (38.8%). Only 14.5% of Jahai were overweight and none were obese, whereas 17.5% of the Kensiu fell into the overweight category. The Jahai and Kensiu tended to have more underweight individuals, comprising 11.6% and 26.8% of the participants respectively.

The participants also provided their dietary information and it was clear that the Temuan dan Bidayuh who live in urban or semi-urban areas consumed three full meals or more, a day. Their diet consisted of carbohydrate, oil and protein rich foods. Meat was eaten almost daily. In contrast, the Negrito individuals ate substantially less and in many cases, only subsisted on one full meal a day. They consumed fish approximately twice a week and ate even less meat. Most of them supplemented their meagre diet with plants and herbs harvested from the surrounding forest.

Blood total cholesterol

The mean blood total cholesterol for all groups was found to be within the normal range if taken collectively, specifically, the Temuan recorded the highest total blood cholesterol mean reading (4.85 mmol/l) followed by the Bidayuh (4.75 mmol/l). Individuals whose blood total cholesterol levels were either lower or higher than the monitor's range (3.88–7.76 mmol/l) were recorded as having 'low' and 'high' levels and thus omitted from the mean calculations. They were, however, included for the percent distribution of total cholesterol levels. The Temuan and Bidayuh again showed high incidences of high total cholesterol levels among four groups, at 23.6% and 19.8% respectively (Table 5.1). Interestingly, a high percentage of the Kensiu (43.9%) and Jahai (54.1%) individuals had extremely low cholesterol levels and were classified as hypocholestrolemic. In some participants, no cholesterol readings were registered on the Accutrend monitor, despite several attempts with fresh blood samples.

Oxidative indices

The malondialdehyde (MDA) levels (measurements of the extent of damage to lipids) in the Temuan and Jahai individuals were high and comparable to the levels in type 2 diabetic patients comprising Malay, Indian and Chinese living in the Klang Valley (Indran et al., 2001). MDA levels in the Bidayuh were generally comparable to the normal healthy population in the Klang Valley (Indran et al., 2001). The Kensiu displayed significantly higher levels of FRAP ($p < 0.05$) and lower levels of MDA when compared to other subgroups, indicating a lower oxidative stress levels.

Interestingly, a strong positive correlation ($p < 0.01$, $r = 0.8$) was observed between MDA and age in Temuan and Jahai individuals. In addition, a strong negative correlation was also observed between FRAP and age ($p < 0.01$, $r = -0.7$); FRAP and MDA ($p < 0.01$, $r = -0.8$) in the Temuan and Jahai group. There was no significant correlation between the various biomedical parameters and the oxidative indices examined in the Bidayuh group. It is note-worthy that the FRAP levels in the Bidayuh were the lowest among the groups. The MDA level is also low and comparable to the normal healthy population residing in the Klang Valley. A comprehensive and more detailed analysis of these parameters will be available for reference (Jinam et al., 2008).

Human leukocyte antigens (HLA)

Analysis of HLA-A and HLA-DQB1 polymorphisms across all groups inclusive of Chinese, Malay and Indian (for comparative purpose), was carried by the SSP-PCR method. A total of 17 HLA-A antigen or allele groups were typed and five predominant alleles or allele groups that were identified were HLA A*02, A*11, A*24, A*33 and A*34. The A*24 allele was most commonly found in Orang Asli (48%), Bidayuh (61%) and Malay (25%). In Chinese, A*02 (32.8%) and A*11 (31.9%) were predominant. In Indians, A*01 (19.5%), A*02 (20%) and A*11(13%) were common. Comparisons with other Asian ethnic groups (HLA 1997 data) revealed that the Bidayuh and Taiwanese aboriginal groups such as the Ami, Atayal, Bunun and Rukai, displayed the highest genotypic frequencies for A*24 ranging from 0.55–0.75.

In the Temuan, Jahai and Kensiu, specific alleles and liked haplotypes that were also identified included A*2407, DQB1*0502, A*3401–B*1521 and A*2407–B*1513. Of the eight HLA-DQB1 allele groups tested, DQB1*05, *0301/4, *03032 and *06 were predominant. DQB1*0305 was absent in all groups except in three Chinese individuals. DQB1*05 was frequently found in the Temuan, Jahai and Kensiu (56.8% collectively), Malay (34.2%) and Bidayuh (33.6%). DQB1*02 (12.8%) and DQB1*04 (5.9%) were highest in the Chinese, while DQB1*06 (43.9%) and DQB*0301/4 (47.6%) were highest in the Indian and Bidayuh, respectively.

The study of HLA Class I and Class II gene diversity showed limited allelic diversity and at the same time revealed very high frequencies of certain allele groups. Possible explanations for these observations include founder effects, genetic drift, population bottleneck events or perhaps selective forces acting on specific alleles. Similarities in allele and associated haplotype frequencies between Orang Asli subgroups indicate admixture and gene flow between those groups while observed likeness with the geographically distant Bidayuh group suggests common ancestry.

The distribution of allele and haplotype frequencies with other populations indicates relatedness with other Asia Pacific groups and a lack of admixture from African and Caucasian populations. These observations agree with population stratification and migration models based on linguistic and archeological data. The data on HLA diversity in these indigenous groups would facilitate disease association studies and increase chances of finding donors for transplantation. Identification of specific HLA alleles using higher resolution typing methods such as sequence-based typing is needed in order to better describe the diversity

and evolutionary events which occurred in these indigenous groups. Data from mitochondrial studies also indicate very early variants suggesting that perhaps the indigenous groups were the first migrants into Southeast Asia (Hill et al., 2006; Hong, 2008).

Malaria

Malaria, which is one of the major infectious diseases in the world, is caused by *Plasmodium* parasite. Many studies have postulated that genetic polymorphisms may influence the susceptibility to malaria. For instance, the genetic variations in haemoglobin S and E, cytokine promoter regions, X-chromosome-linked G6PD gene, as well as ovalocytosis (Fortin et al., 2002), have been studied intensively in relation to malaria. On average, the anti-malaria gene variants are in higher frequency among Orang Asli than other populations in Peninsular Malaysia. These variants are thought to account for up to 48% of all cases recorded in the early part of the 1980s (Baer, 1999).

Ovalocytosis is a common erythrocyte polymorphism in malarious regions. It is manifested by the presence of oval shaped red blood cells with rounded end (elliptocytocis). Glycophorin C exon 3 deletion (GPCΔex3) is associated with Melanesian Gerbich negativity and increased ovalocytosis (Patel et al., 2001). Gerbich negative blood group, which is a rare blood group, is common in Papua New Guinea where malaria is hyper endemic (Serjeantson, 1989). The antigens for the Gerbich blood group system reside on glycophorin C (GPC) or glycophorin D (GPD) glycoproteins. These polymorphisms have been allied with protection against malaria (Maier et al., 2002).

A polymerase chain reaction strategy was employed to genotype individuals for the GPCΔex3 and to determine if GPCΔex3 reduces susceptibility to malaria. Blood smears prepared at the time of blood collection were stained with Leishman stain and examined under light microscopy to screen for the presence of malaria parasites. Examination of white blood cells (WBC) differential counts was carried out with a hematology counter.

No malaria parasites were detected in blood films for all groups and body temperature readings were within the normal range. WBC differential counts revealed that 23% of Jahai and 13% of Bidayuh had low neutrophil counts (< 40%). Low neutrophils have been associated with malnutrition. Individuals who had low neutrophil counts are also at risk for developing serious infections such

as viral infection. A total of 13% of Temuan and 17% of Bidayuh displayed high lymphocyte counts (> 44%) whereas 17% of Kensiu displayed low lymphocyte counts (< 20%). The increase in lymphocyte counts may be indicative of viral infections. Overall, 45% of the participants displayed high eosinophil counts (> 6%) which indicate the occurance of parasitic infections. It was not surprising to note that the majority of the respondents had high eosinophil counts due to the obvious dermatological problems they presented especially the Jahai. These communities are prone to parasitic and microbial infections due to their lifestyles and poor health care facilities.

A total of 15% of Kensiu, 12% of Jahai and 16% of Temuan showed high monocyte counts (> 9%). Monocytes are usually activated during wound healing or during infections such as malaria. High basophil counts (> 1%) was displayed in 14% of Temuan, 27% of Kensiu, 23% of Jahai and 24% of Bidayuh. The increase in basophil counts serve as an indicator of hemolytic anemia, chicken pox or hypersensitive reactions. Until absolute differential counts are determined for each cell type and further tests confirmatory tests are conducted, the authors are unable to make firm conclusions. However, given the limitations, the blood count results are valid and indicative of current and previous infections.

Genotyping results did not reveal the presence of the GPCΔex3 variant. Therefore, this deletion polymorphism that was associated with severe malaria in other populations was not present in the subjects. The authors cannot discount the possibility of asymptomatic *Plasmodium falciparum* infections. It is also possible that other genetics variants that were not tested are involved in the pathobiology of malaria. More extensive investigations are required to shed light on this aspect.

Conclusion

Health among the indigenous groups is still generally poor. This is probably due to lack of awareness of healthy diet, good hygiene practices and a combination of socio-economic factors. Urbanization has brought access to high calorie foods and more sedentary lifestyles without the educational outreach programmes to address and promote healthy eating and living habits. The majority of the indigenous peoples were malnourished and underweight in the past, but they are now overweight and at higher risk for diabetes, hypertension and cardiovascular diseases. The HLA genotyping reveals that they have a small gene pool and may represent population bottlenecks and founder effects. There was no direct evidence

of malaria infection among them, although the biomedical test results suggest that it is present.

This study is the first to document the comprehensive biomedical parameters in these four groups and it has important implications for the health management of the minority indigenous groups in Malaysia. There was also evidence that some individuals suffered the ill effects of alcohol and substance abuse, possibly caused by sense of displacement and hopelessness stemming from the departure of their old ways and self-reliance.

The authors hope that the knowledge obtained from these findings will be useful to guide health care and education programmes, in Malaysia and other countries where applicable. The HLA polymorphisms that exist and their immunological expression may serve as important biomarkers to be considered in conferring disease susceptibility or resistance, forensic investigation and also transplant related compatibilities. In addition, this study has provided basic and essential data for larger and more comprehensive studies to assist in the understanding of human origins, migratory history and health parameters of these populations and their relationships to neighbouring populations in both local and global contexts.

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