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Nasopharyngeal carcinoma in Indonesia: epidemiology, incidence, signs, and symptoms at presentation

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Abstract

Among all head and neck (H&N) cancers, nasopharyngeal carcinoma (NPC) represents a distinct entity regarding epidemiology, clinical presentation, biological markers, carcinogenic risk factors, and prognostic factors. NPC is endemic in certain regions of the world, especially in Southeast Asia, and has a poor prognosis. In Indonesia, the recorded mean prevalence is 6.2/100 000, with 13 000 yearly new NPC cases, but otherwise little is documented on NPC in Indonesia. Here, we report on a group of 1121 NPC patients diagnosed and treated at Dr. Cipto Mangunkusumo Hospital, Jakarta, Indonesia between 1996 and 2005. We studied NPC incidence among all H&N cancer cases (n=6000) observed in that period, focusing on age and gender distribution, the ethnic background of patients, and the disease etiology. We also analyzed most prevalent signs and symptoms and staging of NPC patients at first presentation. In this study population, NPC was the most frequent H&N cancer (28.4%), with a male-to-female ratio of 2.4, and was endemic in the Javanese population. Interestingly, NPC appeared to affect patients at a relatively young age (20% juvenile cases) without a bimodal age distribution. Mostly, NPC initiated in the fossa of Rosenmuller and spreaded intracranially or locally as a mass in the head. Occasionally, NPC developed at the submucosal level spreading outside the anatomic limits of the nasopharynx. At presentation, NPC associated with hearing problems, serous otitis media, tinnitus, nasal obstruction, anosmia, bleeding, difficulty in swallowing and dysphonia, and even eye symptoms with diplopia and pain. The initial diagnosis is difficult to make because early signs and symptoms of NPC are not specific to the disease. Early-age Epstein-Barr virus (EBV) infection combined with frequent exposure to environmental carcinogenic co-factors is suggested to cause NPC development. Undifferentiated NPC is the most frequent histological type and is closely associated with EBV. Expression of the EBV-encoded latent membrane protein 1(LMP1) oncogene in biopsy material was compared between NPC patients of < 30 years old and those of ⩾30 years old, matched for sex and tumor stage. Higher LMP1 expression in patients of <30 years old was observed, which was related to more locoregional progressivity. Increased medical awareness of prevailing early stage signs and symptoms coupled to use of EBV-related diagnostic tumor markers may lead to down-staging and timely treatment to improve survival of patients with this aggressive disease.

Key words Nasopharyngeal carcinoma, incidence, epidemiology, Indonesia

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Nasopharyngeal carcinoma (NPC) is a rare malignancy throughout most parts of the world, with a prevalence usually less than 1/100 000[1,2]. NPC is a highly prevalent malignant disease and a leading cause of death in several regions in southern China, and recently improved registry data indicate medium to high prevalence in other countries in Southeast Asia as well. The Guangdong province in South China has the highest prevalence in the world, with approximately 20 to 40

cases per 100 000 inhabitants depending on the region[3-7]. Earlier work showed that Cantonese "boat people" had the highest incidence of NPC (54.7/100 000)[8]. The data from Guangdong were recently paralleled by findings in native Bidayuh people of Serawak, Malaysia, who also had a high incidence of NPC (23.1/100 000) [9], suggesting that improved diagnosis and registry may reveal additional "hot-spots" of high NPC incidence. Besides southern China, high incidence was also reported among Inuits and other native populations of the Arctic region.

Intermediate incidence rates of NPC are seen in South-east Asia, including Singapore (15/100 000), Malaysia (9.7/100 000), Vietnam (7.5/100 000), Taiwan (7/100 000), and the Philippines (6.4/100 000). This trend also applies in Africa, including the eastern country Kenya (5.4/100 000) and northern countries Algeria, Morocco, and Tunisia (5.1/100 000)[10]. Therefore, epidemiologically, NPC is an interesting cancer because of this defined geographic and racial distribution, pointing to genetic, social, and environmental factors in the etiology of this tumor type. The incidence of NPC in other countries is generally low, and it is therefore considered a rare cancer in populations of the Americas, Japan, Korea, and Europe [1,11-14].

NPC is a frequent cancer in Indonesia, rating as the fourth most common tumor after cervical cancer, breast cancer, and skin cancer, and is the most common malignancy in the head and neck. The disease is 100% related to Epstein-Barr virus (EBV) infection, especially the most common undifferentiated type of NPC (WHO type III)[14,15]. In Indonesia, which has an ethnically diverse population of 225 million people, NPC is prevalent among different native people(s) and presents a major socioeconomic problem, with an overall incidence estimated at 6.2/100 000 or about 12 000 new cases per year[16]. Unfortunately, many of these cases go unregistered due to limited medical awareness as well as the lack of hospital facilities and a nationwide cancer diagnostic and registration system. Virtually all cases of NPC show genetic positivity for EBV, with multiple viral genes being expressed in each tumor cell[8].

EBV was identified by Sir Epstein and colleagues in 1964 in cells cultured from African Burkitt lymphoma tumor explants and the virus was subsequently found to infect more than 90% of the world population though was rarely considered pathogenic[17]. Primary infection usually occurs at childhood and is asymptomatic or presents as a mild inflammation or upper respiratory infection[18]. In Indonesia, 100% of children at 5 years of age are infected with EBV and carry latent virus for life[19]. Delayed primary infection may cause a mild self-limiting illness known as infectious mononucleosis in adolescents and adults[4]. Occasionally, EBV infection may lead to chronic severe and fatal diseases, such as hemophagocytic syndrome and X-linked lymphoproliferative syndrome (Duncan's Syndrome). In humans, EBV infection initiates at the oropharyngeal epithelium upon transmission in the saliva. During this infection, the EBV virus infiltrates and transforms submucosal B lymphocytes that are important for viral latent persistence and further dissemination of the infection to distal epithelial surfaces including the nasopharynx [4]. After primary infection, EBV persists for life as a predominantly latent infection of B lymphocytes, with variable but persistent shedding in the saliva.

EBV is the first human virus to be linked to oncogenesis due to its close association with Burkitt lymphoma and subsequent linkage to the etiology of other lymphoid and epithelial malignancies. In immunosuppressed hosts, such as transplant recipients and untreated human immunodeficiency virus (HIV)-infected individuals, EBV is a major risk factor for the development of lymphoproliferative diseases, which may turn into malignant lymphomas if left untreated [20,21]. In immunocompetent individuals, the virus is associated with classic Hodgkin lymphoma, Burkitt lymphoma, and extranodal B and T/natural killer (NK) cell non-Hodgkin's lymphomas, as well as a few gastric adenocarcinomas and most undifferentiated and poorly differentiated NPCs worldwide. Patients (in developing countries) exposed to chronic inflammation and infection have been reported to be more prone to develop EBV-driven malignancies [21], a situation that might apply to Indonesia as well, but has not been analyzed in any detail yet. EBV is active in the malignant cells of all described tumor types, and each type of tumor has a distinct pattern of EBV gene expression. Individual EBV genes associated with the three main latency programs of EBV can contribute to the malignant phenotype, albeit in a different fashion in distinct tumor types[20,21]. EBV has been classified since 1997 as group 1 human carcinogen by the International Agency on Research on Cancer. Recently, the presence of latent EBV in tonsil epithelial cells was demonstrated[22], providing a basis for understanding the link between EBV and carcinogenesis. The data from that study support the model of dual epithelial-lymphoid tropism for the virus *in vivo*, indicating the possibility that healthy tonsil epithelium may play a role in transmission of the virus as part of the viral life cycle, and suggesting that EBV can play an initiating role in associated epithelial lesions like NPC and oral hairy leukoplakia. Transformation of epithelial cells into a malignant disease by EBV may be enhanced by environmental co-carcinogens, and premalignant dysplasia may progress rapidly into cancer[8,13,14,21].

NPC oncogenesis is not simply a consequence of EBV infection alone. More than 95% of adults in all ethnic groups across the world are healthy carriers of EBV. The transformation of EBV infection into a malignant disease is probably a result of viral reactivation in combination with other (epi)genetic

events, including the development of (multiple) cellular genetic lesions due to environmental carcinogens, food components, possibly combined with genetic immunodeficiencies[8,13,21]. Consistent with this hypothesis is the fact that NPC generally occurs several decades after primary EBV infection and NPC risk persists in first-line offspring [12,18]. On the other hand, many NPC cases are found in children and these cases generally have a more aggressive behavior, also suggesting a more direct role of EBV itself [23,24]. The geographic distribution clearly suggests a role for genetic and/or environmental co-factors. Therefore, the etiology of this disease appears to be multi-factorial[10,13]. EBV infection, environmental factors (especially food), gender, and genetic susceptibility are consistent etiologic factors responsible for the higher incidence of NPC in certain ethnic groups, whereas other factors from air and soil, which depend more on the living environment of these groups, are less consistent. The parallel development of abnormal IgG and IgA antibody responses to EBV lytic antigens, which are characteristically associated with NPC development, most likely is a reflection of this process[18].

Because NPC in Indonesia has not been documented in much detail, we here present our clinical and epidemiologic observations on 1121 Indonesian NPC patients examined between 1995 and 2005.

Case Definition and Historical Analysis

All head and neck (H&N) cancer cases analyzed in this study were obtained from the archives of the Dr. Cipto Mangunkusumo General Hospital, which is a referral and teaching hospital located in the center of Jakarta that treats approximately 600 H&N cancer patients yearly[25]. All cases were histologically classified by standardized biopsy and staged according to the 2002 Union for International Cancer Control (UICC) criteria using clinical assessment and CT scan work-up. From an archive of more than 6000 H&N cancer cases registered between 1995 and 2005, we analyzed 1121 pathologically defined NPC cases treated at our hospital from which sufficient data were available. These cases include Indonesian citizens who are not all permanent residents of Jakarta, but also include patients who come from regional hospitals. As a referral hospital, we treat patients not only from Jakarta and surrounding areas but also from other islands and regions like Sumatra, Kalimantan, and Sulawesi. Almost 90% of the 1121 patients had been primarily diagnosed in our hospital; about 10% were referred from other hospitals in Jakarta and surroundings but were confirmed prior to intake in our hospital. Some patients from more distant underdeveloped rural areas lacked long-term follow-up. For overall pathologic data, we were able to access the combined pathologic database of 13 university hospitals in Indonesia compiled under the supervision of Professors Kurniawan and Cornain at our institute[25].

To obtain adequate data for investigation and follow-up, we selected 213 patients from these 1121 NPC patients as a separate study group. In these patients, more detailed analysis was done, including *in situ* hybridization for EBV-encoded RNA (EBER-RISH) using commercial kits (Dako or Novocastra) to prove EBV involvement. The results from this analysis were consistent with pathologic NPC classification. These patients lived in Jakarta and surrounding areas only, therefore enabling us to evaluate this subgroup more regularly and adequately with laboratory tests, which included determining the DNA viral load in nasopharyngeal brushings as well as whole blood samples and tests for EBV serology for IgA to virus capsid antigen-P18 (VCA-P18) and EBV nuclear antigen 1 (EBNA1)[26-28]. These tests were routinely performed at diagnosis, during treatment, and during follow-up after histological verification. Details of the EBV-related diagnostic results in our patients will be published elsewhere. In a selected group of juvenile and adult cases that were matched for TNM stage and sex and confirmed to be EBV positive by EBER-RISH using commercial reagents, we also analyzed the expression of latent membrane protein 1 (LMP1) using OT21C monoclonal antibody-based immunohistochemistry on paraffin-embedded tissue sections, as described before[29,30].

Results

NPC incidence

From the intake registry in the Ear, Nose, and Throat department at Dr. Cipto Mungunkusumo Hospital, which includes 6000 H&N cancer cases registered between 1995 and 2005, we studied the incidence of individual cancer types, including 1121 cases diagnosed as NPC. The gender distribution among NPC cases showed 789 males versus 332 females. Because of incomplete patient records for the overall H&N cancer cases in the first five years, we could only evaluate the exact prevalence of NPC versus other H&N cancers from the year 2000 onwards (Figure 1). Of all H&N cancer patients treated between 2000 and 2005, including patients from referral centers in rural areas, the prevalence of NPC was around 28.35% (948 of 3344), followed by a 14.35% prevalence for skin cancer and 12.3% for lymphoid malignancies. The yearly incidence varied among tumors but the overall data consistently identified NPC as the most common H&N cancer in our

institute for the 10-year period studied. Consultation with 13 other university hospital-based Ear, Nose, and Throat departments and the related pathologic databases throughout Indonesia confirmed this to be a consistent trend in the entire country (data not shown)[25].

In our cases, we found a similar predominance, with 70.4% male and 29.6% female cases yielding a 2.4:1 ratio. The male:female ratio was relatively stable over the years as shown in Figure 2.

Age distribution

NPC patients from various countries are described with ages ranging from 4 to 91 years, with a peak incidence at 50 to 60 years of age in Chinese populations. Generally, NPC is uncommon in individuals under the age of 20 years (less than 1%), whereas a bimodal age distribution has been described in northern Africa, with 20% of patients being below age 30[31-38]. As shown in Figure 3 and Table 1, the age distribution of NPC patients from our hospital had a peak at 40 to 49 years, and more than 80% of patients were diagnosed between 30 and 59 years of age. We observed a significant number (20%) of juvenile NPC cases, aged under 30 years, without a clear bimodal age distribution. Rather, our data showed a steady increase with age peaking at the fifth decade. For the younger age group, we found an intermediate yet stable incidence of 5 to 12

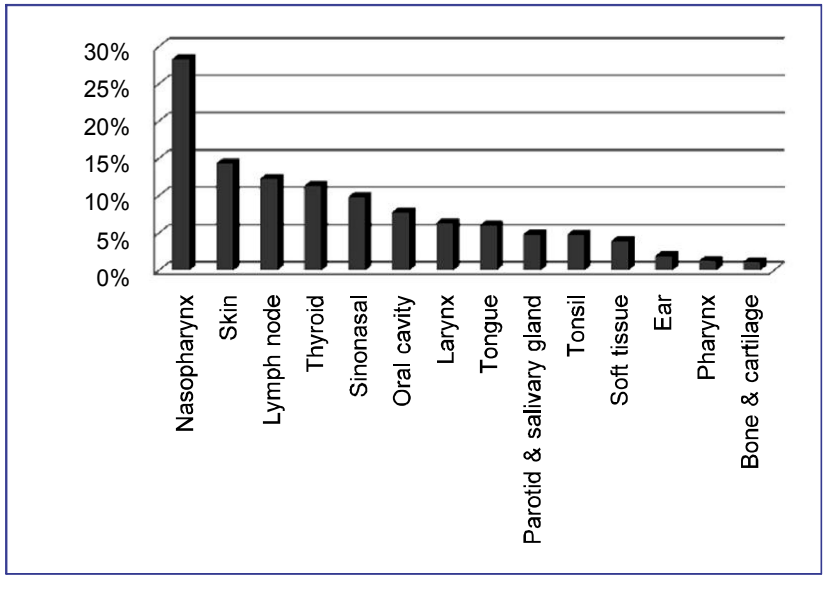

Figure 1. Prevalence of nasopharyngeal cancer (NPC) and other defined malignancies among all head & neck cancer cases (n =3344) examined between 2000 and 2005 in the Dr. Cipto Mangunkusumo Hospital in Jakarta, Indonesia. NPC is the most prevalent head and neck cancer overall, representing about 28% of all cases.

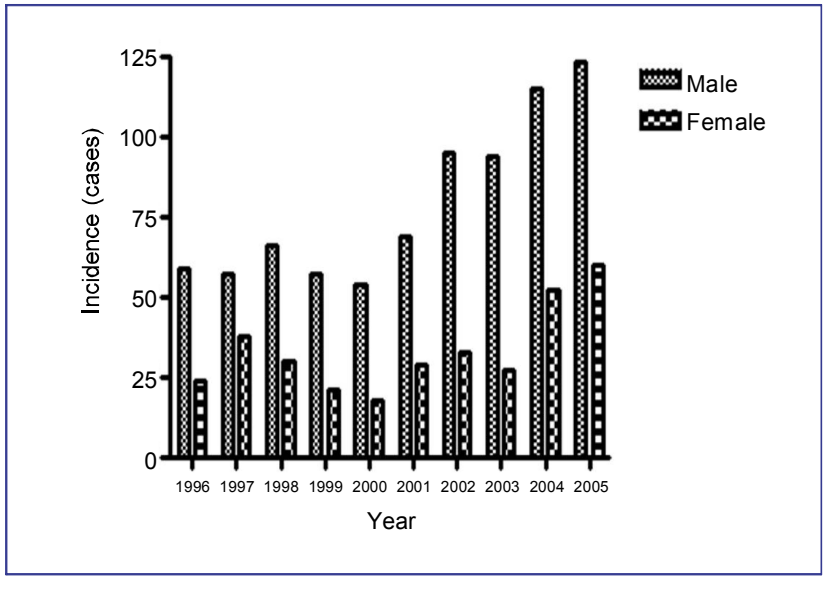

Figure 2. Yearly NPC incidence (total number of NPC cases in the registry per year) and male and female predominance in the 1995–2005 period. The male-female ratio is rather stable over the years with an average of 2.6-fold male predominance. The increase in NPC incidence in recent years (2002 onwards) may be due to improved case definition and increased awareness.

cases yearly (Figure 3 and Table 1). The higher incidence of juvenile NPC may reflect genetic susceptibility and/or young age exposure to co-carcinogens in the environment (to be detailed later).

Regional NPC incidence

Due to the available Pathology Cancer Registry, which includes data from 13 different university hospitals in Indonesia, we were able to investigate the regional incidence of NPC in other areas in Indonesia as well. This registration system is the only access we have to cancer data in our country. Every university center or hospital has its own pathology cancer registration system, part of which is connected to this registry. Based on the data from this registry, we were able to study NPC incidence of 11 pathology centers in Indonesia that could provide sufficient data (Table 2). Because we investigated our subgroup from 1995 till 2005, we chose the year 2000 to evaluate a mean regional incidence. Bandung, Malang, Denpasar, Manado, and Surabaya represented high incidence areas, and therefore, early detection of disease in these cities deserves special attention. Overall, the incidence of 5.66/100 000, equaling roughly 1000 new cases per month, reflected a major health problem in Indonesia, particularly because most of these patients were referred to the hospital at a late stage.

Ethnic origin

Table 3 shows the ethnic origin of our subgroup of 213 patients. Most patients were of Javanese origin, followed by Sundanese and Sumatranese people (30.5% , 25.8% , and 23.9% , respectively), confirming that not only Javanese but also other Indonesian ethnic

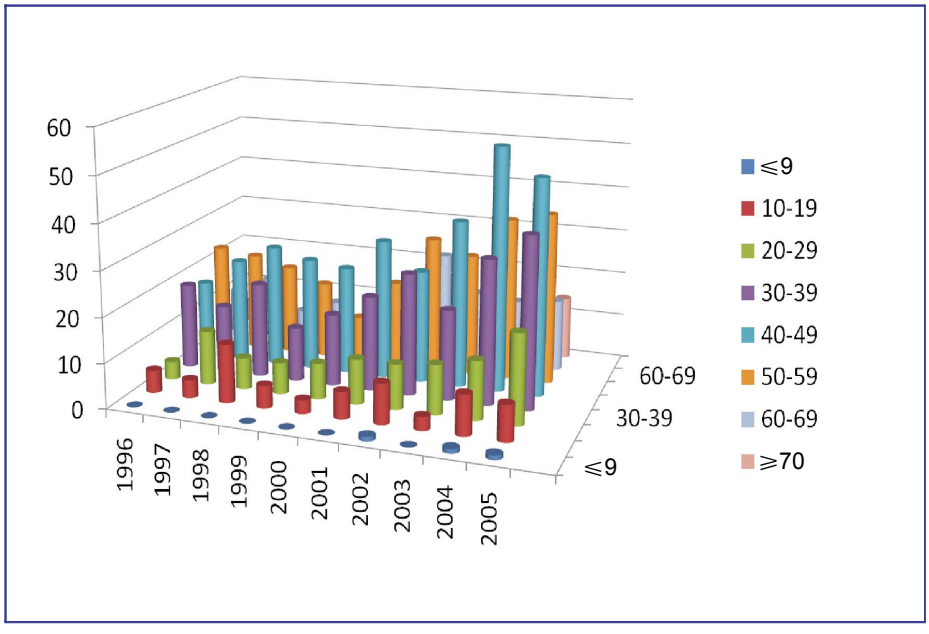

Figure 3. **Age distribution of the 1121 NPC patients in the 1995–2005 period.** Although the NPC incidence in children of <10 years old is low, a considerable number of NPC cases were observed in the age groups 10–19 (Juvenile) and 20–29 (young adult) years old. Overall, the 1–30 year age group represented 21% of all cases. The NPC peak incidence in our cohort lays in the age groups of 30–50 years old, which is earlier than reported for Chinese populations. This illustrates that NPC in Indonesia affects the adult working population who carry responsibility for family and business, thus posing a significant socio-economic burden to the Indonesian society.

Table 1. Age distribution in 213 consecutive local cases of nasopharyngeal carcinoma (NPC) in Dr. Cipto Mangunkusumo Hospital

Age (years)	Frequency	Percentage (%)
<20	21	9.9
21–30	25	11.7
31–40	50	23.5
41–50	69	32.4
51–60	35	16.4
61–70	9	4.2
>70	4	1.9

Table 2. Estimated regional incidence of NPC per 100 000 registered inhabitants derived from 11 of 13 pathology centers in the year 2000

Center (region)	Incidence (/100 000)
Medan	4.30
Padang	2.22
Palembang	3.11
Bandung	7.00
Yogyakarta	4.90
Surakarta	4.93
Surabaya	7.23
Malang	9.19
Denpasar	8.41
Manado	6.54
Jakarta	4.41
INDONESIA	5.66

Two centers were excluded because of too little supportive data.

Table 3. Distribution of ethnic origin of 213 NPC patients from the Jakarta region

Ethnicity	Frequency	Percentage (%)
Java	65	30.5
Sunda	55	25.8
Sumatera	51	23.9
Betawi	25	9.9
Others	20	8.0
Chinese	4	1.9

groups were affected by NPC. More recent data for the 2007–2011 period from an ongoing treatment study at the radiotherapy department in our hospital confirmed that Javanese were the most prevalent ethnic group being treated for NPC (375/1173 patients, 32%), followed by Sundanese (19.2%), Chinese (10.6%), Batak (9.5%), Betawi (7.6%), Lampung (2.9%), and Minangkabau (2.4%) groups (Soehartati G, *et al.*, personal communication). Although a strong genetic control is suggested for the disease, the incidence pattern among different ethnic groups in the Indonesian population showed no marked differences. For example, Chinese migrants have been shown to retain a high incidence of NPC even in subsequent generations, suggesting a strong genetic control. Our data reveal that in Indonesia, the disease does not follow Chinese demographics and does not seem to be influenced by Chinese genetics despite the large population of Chinese descendants living in Jakarta and its surroundings, and despite that a high NPC incidence with Chinese origin is indicated in neighboring countries, including Malaysia and Singapore.

Histopathology

According to WHO classification, NPC is histopathologically divided into three categories: keratinizing squamous cell carcinoma (WHO type I), non-keratinizing squamous cell carcinoma (WHO type II), and undifferentiated carcinoma (WHO type III). NPC WHO type III, is the most prevalent form of NPC in Southeast Asia and other high incidence regions, and is most closely associated with EBV infection. WHO type I tumors can also be associated with EBV in endemic regions, but usually not in non-endemic regions, where they result from tobacco and alcohol abuse and are EBV-negative[39,40].

WHO type III was the most frequent histopathologic type in our study population. Around 85.0% of cases proved to be undifferentiated carcinoma. Interestingly, our population contained 12.7% WHO type I NPC tumors (classification confirmed via blind reassessment by independent pathologists), all of which were EBV-positive by EBER-RISH. Only 2.3% of the cases

were classified as WHO type II NPC tumors.

In our selected 213 cases, we also studied the differences of EBV-LMP1 expression in NPC between patients of < 30 years (n = 24) and ≥ 30 years old (n = 24), matched for sex and tumor TNM stage (Table 4). LMP1 expression was detected in 160 (75%) cases with a staining intensity score ranged from 0 to 11.7. The average score for patients of < 30 years old was higher, but was not different significantly (P > 0.05). There was a borderline significant relationship between LMP1 expression and T stage (P = 0.042), but not with N and M stages. The intensity score of EBV-LMP1 expression in this study was somewhat lower than others[30]. Higher LMP1 expression in patients < 30 years old was associated with more locoregional progressivity at young age.

Etiology

Early age EBV infection and chronic viral reactivation in nasopharyngeal epithelial tissues due to locoregional inflammation may be fundamental for NPC development. In this respect, it should be noted that nearly 100% of Indonesian children are EBV carrier at age 5[19]. Many environmental factors are considered important for NPC development. Dried salted fish, common in the Indonesian diet, have been reported to cause NPC due to the nitrosamine content[10,41,42]. Chronic exposure to and intake of chemical carcinogens, formalin and phorbol esters, that are also widely spread in Indonesia, are considered as important risk factors as well, although little detail is known yet[43,44]. A reflection of chemical co-carcinogenesis may presented by high levels of genome methylation, as recently described in Indonesian NPC patients and regional controls[45]. A number of studies have reported familial linkage for NPC risk, suggesting genetic susceptibility. However, in our 1121 NPC cases, we did not find any familial association. A number of reports have suggested a role for histocompatibility complex (HLA), in combination with

Table 4. Expression of Epstein-Barr virus-encoded latent membrane protein 1 (LMP1) in juvenile and adult NPC cases

Item	Cases	Frequency of LMP1 expression			P
		Mean ± SD	Median	Range	
Age					0.795
<30 years	24	2.9 ± 4.0	0.7	0 – 11.7	
⩾30 years	24	1.8 ± 2.3	0.8	0 – 8.4	
Sex					0.950
Male	28	2.7 ± 3.8	0.6	0 – 11.7	
Female	20	1.9 ± 2.5	0.9	0 – 8.4	
WHO histopathologic type					0.364
WHO I	8	2.8 ± 2.9	2.5	0 – 8.4	
WHO III	40	2.3 ± 3.4	0.6	0 – 11.7	
WF histopathologic type					0.364
KS	8	2.8 ± 2.9	2.5	0 – 8.4	
Type A	40	2.3 ± 3.4	0.6	0 – 11.7	
Analysis stage					0.589
Early	8	1.0 ± 1.2	0.8	0 – 3.6	
Advanced	40	2.6 ± 3.5	0.7	0 – 11.7	
T stage					0.042
T1	6	1.8 ± 1.6	1.85	0 – 3.6	
T2	15	2.5 ± 3.6	0.8	0 – 10.5	
T3	9	1.4 ± 0.8	0.1	0 – 4.4	0.039 (T1–T3)
T4	18	3.6 ± 4.0	1.7	0 – 11.7	0.007 (T3–T4)
N stage					0.553
N0	4	2.0 ± 3.8	0.1	0 – 7.6	
N1	12	1.8 ± 2.7	0.9	0 – 9.6	
N2	12	1.7 ± 2.3	0.5	0 – 7.8	
N3	20	3.2 ± 4.1	0.8	0 – 11.7	
M stage					0.706
M0	42	2.4 ± 3.4	0.8	0 – 11.7	
M1	6	1.7 ± 3.1	0.6	0 – 8.0	

LMP1 expression level was assessed on paraffin tissue sections with OT21C mouse anti-LMP1 monoclonal antibody staining, using the scoring method as detailed elsewhere[30].

EBV mutant strains, in NPC. HLA linkage data reveal that younger and older onset patients are genetically different and may involve different mechanisms [10-12]. Recent genome-wide linkage analyses of high-risk Chinese familial NPC pedigrees identified two candidate NPC susceptibility loci, 4p15.1-q12 and 3p21.3, with another suspected locus reported at 5p13-15 [8,46-54]. However, more recent large-scale studies with appropriate local non-NPC controls have cast doubt on these early findings, and no clear NPC-related EBV strain nor (limited number) genetic marker has been identified as outstanding entity[51].

Clinical signs and symptoms at presentation

Most patients in our study cohort presented with advanced disease. Early stage NPC is difficult to diagnose clinically because of its hidden localization in the nasopharynx. Misdiagnosis could also result from patients who lack of knowledge about early signs and symptoms of NPC and cancer in general. Denial of cancer diagnosis and economical restrictions may delay medical treatment. On the other hand, doctors also contribute to late NPC diagnosis because of ignoring or misdiagnosing the unspecific symptoms mimicking upper respiratory tract infection during early stages. A recent study confirmed the poor awareness of NPC early signs and symptoms among regional health workers in Indonesia[55]. Basically, examination and biopsies of the tumor and the nasopharynx need to be performed by a direct nasoendoscopic examination (preferably using flexible fibreoptic endoscope). This is one of the most important skills required for the diagnosis and monitoring of NPC and may facilitate accurate brush sampling in the nasopharyngeal space to assess EBV-DNA load, which appears closely linked to local presence of NPC[27]. In high-risk regions, doctors should be more aware of early-stage, unspecific signs and symptoms to improve recognition, diagnosis, and downsizing of tumors at presentation, thereby improving treatment options. With this in mind, we ranked the most common signs and symptoms of Indonesian NPC patients in our study, by compiling the results from a questionnaire completed on patient intake (Figure 4). Most of our patients (60.6%)

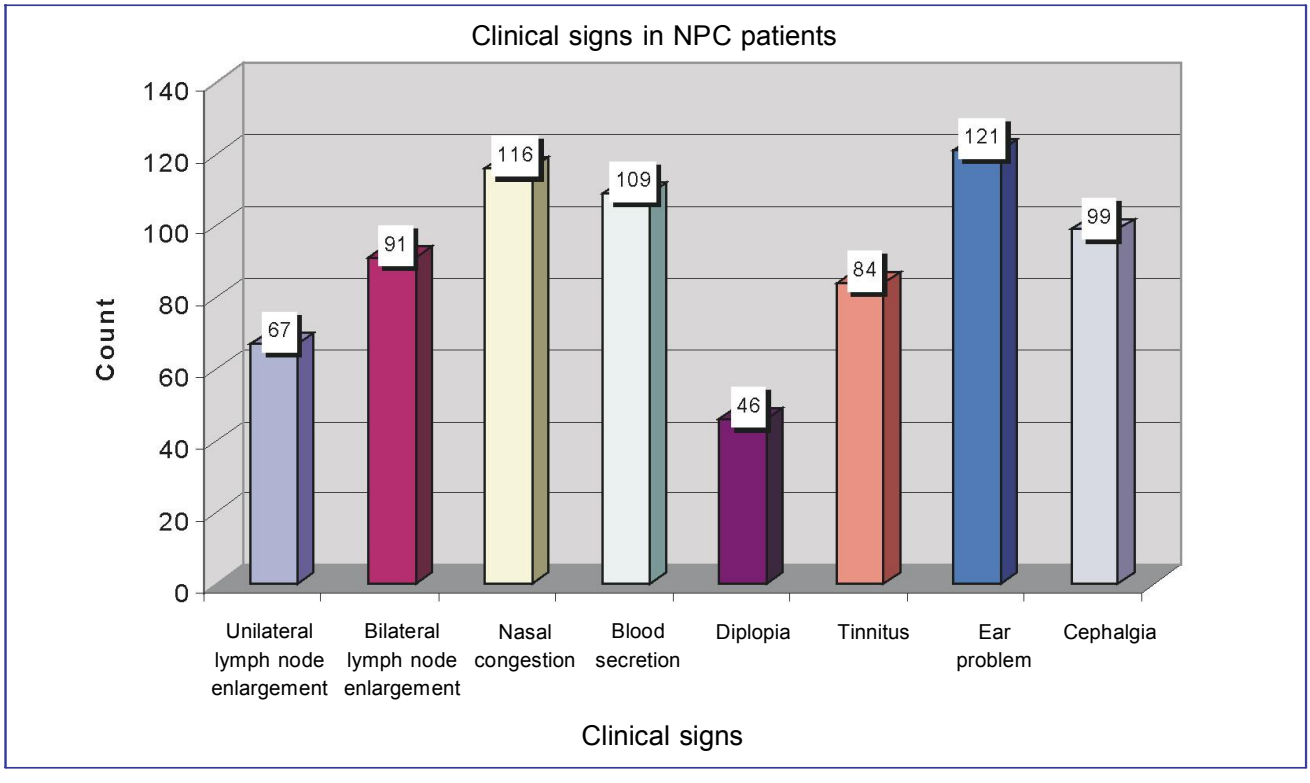

Figure 4. **Most predominant signs and symptoms at first presentation in 733 of the 1121 NPC patients.** It may be deduced from this graph that most common symptoms associating with first presentation are generic for many other ear, nose, throat syndromes and cannot be taken as being characteristic for NPC. However, doctors confronted with patients having a combined or chronic history of these symptoms, without relief by conventional (anti-bacterial, anti-allergic) therapy, should be on alert for more detailed investigation at early stage, including nasendoscopy and EBV-IgA serology [28]. Upon persisting symptoms and abnormal positive EBV-IgA serology novel non-invasive diagnostic procedures, like nasopharyngeal brushing combined with EBV-DNA measurement [27], may be indicated and informative for early detection of NPC as underlying cause of symptoms.

had recognized they already had a unilateral ear problem, the earliest sign of NPC, several months before diagnosis. Second and third most prevalent symptoms at presentation were persistent nasal congestion and nasal blood secretion. However, our data indicated that neither patients nor doctors gave this condition attention until cervical lymph node enlargement, a sign of late stage NPC, was detected.

Discussion

Here, we presented our data about the incidence of NPC in Indonesia using pathologic reports from 13 university centers in Indonesia collected in the Pathology Cancer Registry System. We also evaluated, in Dr. Cipto Mangunkusumo Hospital, NPC incidence in all patients treated between 1995 and 2005 and assessed the epidemiologic and clinical data of 213 patients in which additional markers for NPC were examined. Overall, our data on NPC prevalence for Indonesia are comparable to those reported in 1998 by Soeripto [16], indicating a prevalence of around 6 /100 000, which is in line with the Globocan-IARC estimates as reported[1,11].

From the above mentioned data, it is clear that Indonesia is still an unexplored region with considerable NPC incidence, yielding about 12 000 new NPC cases on a yearly basis. Denpasar, Malang, Surabaya, and Bandung are, for example, regions of high incidence. Acquired data for these regions are poor in detail, suggesting these regions should be explored more intensively. Although most patients in our study are of Javanese origin, it became clear that other ethnic groups in the overall population of Indonesia are also affected by NPC. Importantly, upperclass Indonesian patients may seek specialized treatment in neighboring countries like Singapore, Malaysia, and China. It is appreciated that these may include patients of Chinese origin, but this is unlikely to influence the overall results on ethnic origin. Therefore, NPC is a major multi-ethnic problem in Indonesia and not only linked to Chinese genetics. A prior study by Devi *et al.* [9] in the province of Sarawak, Malaysia showed that the age-adjusted incidence in Sarawak residents was 13.5/100 000 (95% CI: 12.2–15.0/100 000) in males and 6.2/100 000 (95% CI: 5.7–6.7/100 000) in females. The risk in the Bidayuh people was 2.3-fold (males) and 1.9-fold (females) higher than the Sarawak average and about 50% higher than those in other regional populations. The high risk in native people of Sarawak, however, is unlikely to result from blending with citizens of Chinese descent that form a distinct ethnic group in Malaysia, similar to Singapore. These findings and data from this study suggest NPC risk to be endogenous to the local population in Southeast Asian multi-ethnic countries, including Indonesia. The observed increase of NPC cases in our institute in recent years may be due to improved referral rather than true incidence. This may be related to improved awareness and implementation of more advanced treatment options, in particular in the Jakarta region.

The age distribution of NPC in Indonesia is different compared to previous data from China and North Africa. A similar age distribution has also been reported by Loh *et al.* [36], who showed that of 323 new patients treated between 1998 and 2004 in the National University Hospital in Singapore, 36% to 40% were diagnosed at 41 to 50 years of age. In the literature, an overall peak incidence is described at 50 to 60 years of age. In high risk areas, such as Hong Kong, the NPC incidence in each sex rises sharply from the age of 20 onward and also reaches a plateau between 40 and 60 years of age [33]. In addition to this peak incidence at middle age, a second peak incidence is described in the literature for a younger age group, 10 to 29 years. This peak incidence is particularly found in northern African countries and some Chinese populations as well [37,38]. In China, the overwhelming majority of the cases occur in the fifth and sixth decades of life. In contrast, there is a bimodal distribution in North Africa, with a major peak incidence around 50 years of age, similar to the single peak observed in China, and a minor peak in people aged between 10 and 25 years old. This juvenile form accounts for approximately 20% of the patients and has specific clinical and biological features[3]. Jeannel *et al.*[10] reported that the age-specific incidence for NPC differs from other tumor types affecting older age groups. Whereas the peak incidence for other tumors is reached around the age of 45 to 49 years, the incidence of NPC is approximately stable until 60 to 64 years of age, after which it declines. In Indonesia, a steady increase is observed well before the age of 45, starting at early adolescence. Age distribution for NPC is bimodal in some northern American populations and in the Mediterranean region, with a peak incidence at 10 to 20 years and a second at 40 to 60 years of age. Children under 16 years of age account for 1% to 2% of all patients with NPC in China, 2.4% in the United Kindom, 7.12% in Turkey, 10% in the United States, 12% in Israel, 13% in Kenya, 14.5% in Tunisia, and 18% in Uganda[14]. In Indonesia, 17% to 21% of all patients are under the age of 30, as observed over a 10-year period. Our data on the overall NPC incidence did not differ significantly among regional centers in the larger Jakarta area and were also similar to those obtained in the Dr. Sardjito Hospital at the Gadjah Mada University in Yogyakarta, a more rural region of Mid-Java, where 450 cases were recently analyzed (Hariwiyanto B; unpublished data and personal communication).

Previous epidemiologic studies suggest three major etiologic factors for NPC: genetic susceptibility, early age

exposure to chemical carcinogens (particularly Cantonese salted fish), and latent EBV infection [7,8,41,42]. Preserved foods other than salted fish could also play a part in the etiology of NPC and methods of cooking may have an effect on the amount of volatile nitrosamines ingested [41]. In Malaysian Chinese, the consumption of beef liver, in addition to salted fish and salted eggs, appeared significantly associated with NPC. In addition to these factors, the presence of nitrosodiethyl amine in smoke and dried meat and the use of herbal nasal medicine are well-known risk factors of NPC. Also, improperly (formalin-treated) preserved foodstuffs, which are rather common in Indonesia[43,44], may be important as etiologic risk factors. Another risk factor is environmental inhalants, a significant number of which have been reported to be associated with NPC. These include fossil fuels from cooking due to smoke and fumes from wood, which contains significant quantities of benzopyrene, benzanthracene, and polycyclic aromatic hydrocarbons. Another source of carcinogenic hydrocarbons is textile dyes, which are still in common use in local Indonesian markets for food coloring. The consumption of some herbal teas, and in particular teas containing Euphorbia family plant extracts, is considered a risk factor. Occupational exposure of formaldehyde also increases the risk. Finally, smoking cigarettes with exotic additives and working in poorly ventilated places are strongly associated with NPC. Interestingly, the widely spread use of incense burning in Southeast Asia has not yet been considered as a risk factor.

Preserved vegetable intake is associated with a 2-fold increase in NPC risk, whereas high non-preserved vegetable intake is associated with a 36% decrease, consistent between vegetable types and countries. Direct measurements of N-nitroso compounds from preserved foods collected in regions of high and low incidence as well as in different areas within a high incidence region did not correlate with the regional and local variations in incidence. In contrast, preserved and fresh foods consumed in developing and Western countries contain very low levels of N-nitroso compounds [10,13]. The content of N-nitroso compounds in Indonesia has not been evaluated yet.

Epidemiologic studies point to the protective role of regular consumption of fresh fruits and vegetables, presumably because of the vitamin content, especially vitamin C. Vitamin C may act in blocking either nitroso compound metabolism or EBV reactivation. Activation of EBV *in vitro* by tumor promoter TPA (12–0 tetradecanoyl phorbol-13-acetate of the phorbol ester family), which has EBV lytic cycle-inducing capacities, can be inhibited by vitamin C [10,37]. Furthermore, besides the widespread consumption of dried salty fish, it is rather common in Indonesia to find known carcinogens like formalin and polyaromatic chemical dyes in the food supply at local markets and small factories [43,44]. Furthermore, cigarette smoking and "therapeutic" inhalation of various aromatics are rather common in Indonesia, adding to the co-carcinogen burden from the environment. Chronic exposure to these (co-)carcinogenic factors and EBV latent infection may increase, in synergy, the risk for NPC development. Chronic exposure to co-carcinogenic compounds may be reflected in increased methylation of defined tumor suppressor genes, as recently revealed by us and others[45,50].

EBER *in situ* hybridization (EBER-RISH) is considered the gold standard for detecting and localizing latent EBV in tissue specimens, whether frozen or formalin-fixed and parafin-embedded[56,57]. This test is the most reliable method for determining if a lesion is EBV-associated and is used diagnostically in several specific clinical situations. In biopsy, EBER-RISH is often helpful in differentiating infectious mononucleosis, Hodgkin's disease, and/or non-Hodgkin's lymphoma and to define EBV involvement in the pathogenic process. It is also used routinely for confirming a diagnosis of EBV-driven postransplant lymphoproliferative disorder (PTLD) [58]. Further analysis using EBER-RISH is warranted to define the overall impact of EBV involvement in Indonesian H&N cancers including NPC. However, EBER-RISH is an expensive and complex procedure, not well suited for routine application under sub-optimal laboratory conditions[56]. Likewise, a biopsy from the nasopharyngeal space is a painful and invasive procedure and tissue processing may not be generally available. Therefore, current efforts are on defining non-invasive diagnostic procedures based on EBV-DNA detection in blood, plasma, or nasopharyngeal brushings [26-28,56]. Additionally EBV-IgA serology may prove suitable for early identification of individuals at risk (family members) or at early stages of NPC[36].

These novel approaches are becoming increasingly available and may ready for large scale (screening) in the near future, which will be of particular relevance to developing countries with medium-high NPC incidence like Indonesia.

NPC is ranked fourth among cancers in males in Indonesia. Patients are generally referred at a late stage. The overall treatment is complex, not cost-effective, and places a significant socio-economic burden onto patients and their families. Adequate data for follow-up from referral centers are usually not available. Registration of patients with NPC is, in most cases, not digital and therefore inadequate. Thus, it is difficult to compare treatment results from several centers and even more difficult to compare treatment results with other countries or to include patients in protocols for international studies. Patients are often referred to the hospital at a late stage, which has a major drawback on their prognosis. As a result, even many young patients are

treated for late-stage disease and unfortunately become victims of a deadly disease at a young age. There is no doubt that this disease, affecting individuals at 40 to 50 years of age as well as those under 30 years old, represents a large socio-economic burden for the country and its health system. Therefore, early detection by simple and affordable techniques, such as nasopharyngeal brushing[27] and blood investigations[26,28] and adjuvant laboratory examinations, for regular assessment of the status of disease-specific markers, are of utmost importance. Molecular testing, such as peptide-based EBV-IgA serology and EBV-DNA load testing, holds promise for early detection and down-staging NPC in Indonesia when applied on a country-wide scale. The availability of simple sampling and stabilized transport options is relevant for collection of clinical specimens at remote (rural) health centers[59]. Finally, the need for and importance of adequate digital early registration of patients for treatment and follow-up of NPC also cannot be overestimated.

NPC remains one of the most confusing and commonly misdiagnosed diseases. There are multiple non-specific early signs and symptoms of NPC, but they can be taken as early warnings for doctors to improve awareness and send samples for specific testing. Educating regional health workers and hospital staff is a critical first step for controlling NPC at early stage.

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