Poor Prognosis in Nasopharyngeal Cancer Patients with Low Glucose-6-phosphate-dehydrogenase Activity

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Nasopharyngeal carcinoma (NPC) is endemic among well-defined ethnic groups in several world regions, such as Southeastern China and Taiwan. Glucose-6-phosphate-dehydrogenase (G6PD)-deficiency, a sex-linked disorder, is one of the most common enzymopathies in Taiwan. The major role of G6PD is to generate NADPH to protect cells from oxidative damage, which is a major contributing factor to certain degenerative diseases, such as aging and cancer. In view of the coincidence of epidemic distribution of NPC and G6PD deficiency, as well as the house-keeping function of G6PD in cellular oxidative defense, we investigated the correlation of G6PD activity with NPC. The stage of NPC was classified by AJCC (1997) criteria. G6PD levels were determined in 108 NPC male patients and 75 healthy male individuals. The mean G6PD level of NPC patients was 218.9 U/10^{12} RBC or 7.53 U/g hemoglobin (Hb), being much lower than in normal individuals (260.6 U/10^{12} erythrocytes (RBC) or 8.92 U/gHb). The level of G6PD activity had no correlation with tumor stage or lymph node or distant metastasis, but was significantly correlated with tumor recurrence ($P=0.004$ when using G6PD=130 U/10^{12} RBC as cutoff value). These results indicated that low G6PD activity in patients with NPC is associated with poor prognosis.

Key words: Nasopharyngeal cancer — G6PD — Oxidative defense — Epstein-Barr virus

Nasopharyngeal carcinoma (NPC) is a cancer of epidermoid origin with an endemic distribution among well-defined ethnic groups in several world regions. Southeastern China and Taiwan have the highest incidence (10 to 80 per 100 000 persons per year), followed by North Africa, the Philippines, and the Caribbean nations, but the tumor is rare in Caucasians.[1, 2] NPC occurs more frequently in males, at a rate approximately 3-fold higher than in females.[2, 3] Aside from the striking gender and racial predilections of the disease, other factors implicated in the development of NPC are the Epstein-Barr virus (EBV), and genetic and environmental factors. EBV DNA is present in almost every NPC specimen. However, there is compelling evidence that the virus causes a subclinical infection early in life, resulting in over 90% seropositivity of susceptible individuals by 3 years of age in the Far East.[4, 5] These facts indicate that EBV may be an essential but not a sufficient factor for the development of NPC.[5] The fact that populations in Southeastern China and Taiwan are preferentially affected by NPC suggests a genetic predisposition for the disease. Several studies of genetic systems and karyotypes have found that HLA-2 and HLA-BS, as well as the deletion of chromosomes 3p and 9p, are often associated with NPC.[6–8] Although these studies have suggested possible sources of carcinogenesis, little is known about the malignant progression of NPC.

Glucose-6-phosphate-dehydrogenase (G6PD), the first enzyme in the hexose monophosphate shunt, generates NADPH, a major reducing agent within cells.[9] Oxidative stress has been proposed as a pathological contributor to many diseases, particularly degenerative disorders such as cancer.[10–12] If oxidative damage is one of the contributing factors to the pathogenesis of NPC, patients with a defective antioxidant system should be more prone to oxidative damage and hence, to accelerated cancer development. G6PD-deficiency is a common sex-linked disorder found in Southeastern China (5.5% in Guangdong province) and Taiwan (3%).[13, 14] Since G6PD is a very important house-keeping enzyme to provide reducing equivalents against oxidative damage in all cells, and there exists such a high coincidence of epidemic distribution between NPC and G6PD-deficiency, we investigated the relationship between G6PD activity and NPC in male patients.

MATERIALS AND METHODS

Patients and samples   After informed consent had been obtained, blood specimens were collected. Blood speci-

mens were collected in tubes containing anticoagulant from 108 male patients with nasopharyngeal carcinoma who consecutively visited the Otorhinolaryngology or Radiation Oncology Clinic at Chang Gung Memorial Hospital (Taoyuan, Taiwan) from March 1996 to August 1998. All of the main clinical and pathologic characteristics as well as the treatment procedures were carefully recorded in hospital charts. The median age of the 108 NPC patients was 46, ranging from 24 to 81. All patients showed good performance status (WHO score <2). No patient had distant metastatic disease. The distribution of tumor staging classified by the 1997 AJCC system[15] into T1, T2, T3 and T4 was 40, 14, 28 and 26 patients, respectively. The distribution of overall stage was 6, 39, 29 and 33 patients in stage I, II, III and IV, respectively. The median follow-up time was 24.8 months, ranging from 6 to 37 months.

The control group consisted of sex and age-matched individuals with no history of degenerative diseases, such as cardiovascular disease, diabetes or cataract, who were selected from the physical check-up department at the Chang Gung Memorial Hospital. At least one matching control specimen was obtained at the same time whenever specimens from NPC patients were available. Blood specimens from 75 healthy individuals were collected. The mean age of the control group was closely matched with the mean age of the NPC group. All of the samples were assayed within 24 h of collection.

Treatment for NPC patients The treatment protocol was relatively uniform for the 108 NPC patients. The radiation treatment method has been described in detail previously.[16, 17] In brief, the initial irradiation treatment was 6 MV X-rays, using bilaterally opposed portals to the primary tumor and upper neck, and anterior portals to the low neck. The daily dose was 2 Gy, and five treatments were given per week. After 46 Gy, the primary tumor was boosted with 10 MV X-rays using bilateral fields to 56 Gy to spare the spinal cord. The 3-D conformal irradiation technique was used to apply 64 Gy to T1 and T2a tumor, 72 Gy to T2b and T3 tumor, and 76 Gy to T4 tumor. High dose rate brachytherapy with Ir-192 isotope was delivered to T1 and T2a tumor for another 8 Gy in 4 fractions. The total irradiation dose to T1–T3 lesion was 72 Gy and that to T4 lesion was 76 Gy. In addition, 8–10 MeV electron beam irradiation was applied to the posterior and lower neck area for 24 Gy/12 fractions to the initial gross neck tumor area and 14 Gy/7 fractions to subclinical but high-risk areas. The total radiation dose to the gross neck lymph node was 70 Gy, with 60 Gy to high-risk areas.

Two courses of induction chemotherapy were given to N2 and N3 patients, while concurrent weekly chemotherapy was given to T3 and T4 patients. The chemotherapy was cisplatin-based agents.

G6PD activity assay The G6PD activity in fresh blood cells was quantitatively measured using a No. 345-B kit supplied by Sigma as previously described.[18] All of the assay procedures followed the standard protocols in the manufacturer's instructions. G6PD activity is expressed as $U/10^{12}$ erythrocytes (RBC) or as U/g hemoglobulin (Hb).

Statistical analysis Mean, standard deviation (SD), frequency, percentage and Kaplan-Meier rate were used to summarize the data. Two-sample unpaired t test or analysis of variance (ANOVA) was used to compare continuous data among groups where appropriate. The effects on recurrence or metastasis of the possible prognostic factors (age, T stage, N stage, G6PD activity [$U/10^{12}$ RBC or U/g Hb]) were examined univariately using the log-rank test, and multivariately using Cox's proportional hazard model. All reported P values were two-sided. A difference was considered statistically significant when P was less than or equal to 0.05.

RESULTS

G6PD activity in healthy adults and patients with NPC The mean value (±SD) of G6PD activity in 75 healthy male adults was 260.6 ±84.42 per RBCs ($U/10^{12}$ cells) or 8.92±2.97 per hemoglobulin (U/g Hb). The mean value of G6PD activity in 108 NPC patients was 218.9±67.93 per RBCs ($U/10^{12}$ cells) or 7.47±2.41 per hemoglobulin (U/g Hb) (Table I). G6PD activity in NPC patients was significantly lower than that in normal adults ($P<0.001$), suggesting that individuals with low G6PD activity may be more prone to the development of NPC than individuals with normal G6PD activity.

G6PD activity and other prognostic factors in NPC patients Fig. 1 displays the distribution of G6PD activity measured as $U/10^{12}$ RBC and U/g Hb in the NPC patients. The correlation coefficient (r) is 0.891, indicating G6PD activity can be represented by either measurement. G6PD activities in different T or N stages of NPC patients are shown in Tables II and III. There was no significant difference of G6PD activity with the advancement of T or N stages. Association of G6PD with cancer recurrence or metastasis was next examined. The log-rank test was used to separate the G6PD activity into two groups using various cutoff values. G6PD activity below 130 $U/10^{12}$ RBC

Table I. Distribution of G6PD Activity in Healthy Male Adults and NPC Male Patients

Persons	NPC patients	Healthy adults	P value
Number	108	75	
RBC ($U/10^{12}$ RBC)	218.92±67.93	260.6±84.4	<0.001
Hb (U/g Hb)	7.47±2.41	8.92±2.91	<0.001

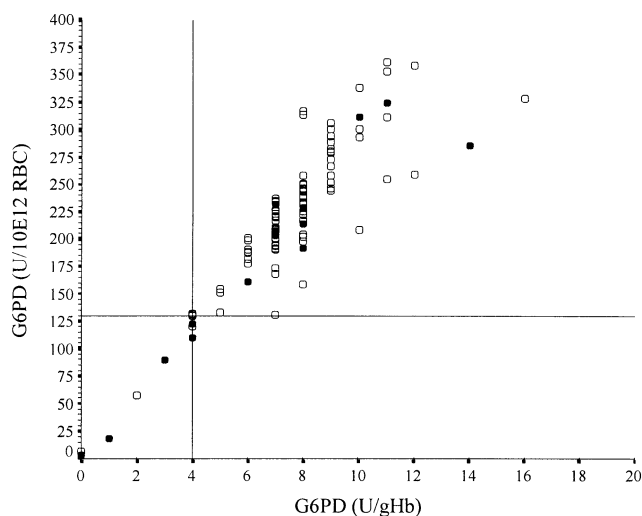The results are shown as mean±SD.

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Fig. 1. Distribution of G6PD activity in NPC patients. In the figure, ■ indicates those NPC patients with cancer recurrence, and □ indicates those NPC patients without cancer recurrence. The correlation coefficient of G6PD activities measured with the two methods is 0.891. Log-rank test was used to separate the G6PD activity into two groups using various cutoff values. G6PD activity below 130 U/10^{12} RBC or 4 U/g Hb showed the most significant difference in recurrence of NPC.

or 4 U/g Hb showed the most significant difference in recurrence (Fig. 1). However, a suitable cutoff of G6PD level to differentiate metastasis was not found.

The association of NPC recurrence or distant metastasis with putative prognostic factors, including the level of G6PD activity, was evaluated. Results of univariate analysis for NPC recurrence and metastasis are listed in Tables

IV and V. As shown in Table IV, NPC recurrence was not correlated with patient's age or tumor T or N stage, but was significantly associated with level of G6PD activity. The 2-year recurrence-free rate was 50% for NPC patients with G6PD activity equal to or less than 130 U/10^{12} RBC, whereas it was 90% for NPC patients with G6PD activity higher than 130 U/10^{12} RBC. The difference was statistically significant ($P=0.0004$). Similarly, the 2-year recurrence-free rate was significantly different ($P=0.0082$) with a G6PD level equal to or less than 4 U/g Hb (60%) versus higher than 4 U/g Hb (90%). Distant metastasis of NPC, however, showed no significant association with any prognostic factor examined (Table V). Results of multivariate analysis for NPC recurrence and metastasis are shown in Tables VI and VII. Again, G6PD activity was associated with NPC recurrence ($P=0.011$) but not with NPC metastasis ($P=0.538$).

DISCUSSION

Cancer development is recognized as a micro-evolutionary process that involves multiple events.[10] These events can be simplified into a three-stage model: the induction of DNA mutation in a somatic cell (initiation), the stimulation of tumorigenic expansion of the cell clone (promotion), and malignant conversion into cancer (progression). Reactive oxygen species (ROS) may be involved in any of these stages and stimulate cancer development.[12, 15] ROS have been shown to damage DNA and protein, inactivate enzymes, peroxidize lipids and perturb membranes. Accumulation of DNA damage may result in the mutation of genes, such as oncogenes and tumor suppressor genes, leading to abnormal control of cell growth. On the other hand, direct oxidative damage to protein and lipid can lead

Table II.   G6PD Activity in NPC Patients at Different T Stages

Stage	T1	T2	T3	T4	P value
Number	40	14	28	26	
G6PD (U/10^{12} RBC)	230.2±53.9	221.3±73.0	212.0±56.0	207.8±56.0	0.559
G6PD (U/g Hb)	7.8±2.1	7.7±2.3	7.3±1.8	7.0±3.7	0.521

The results are shown as mean±SD.

Table III.   G6PD Activity in NPC Patients at Different N Stages

Stage	N0	N1	N2	N3	P value
Number	34	47	16	11	
G6PD (U/10^{12} RBC)	216.1±76.7	218.7±59.6	211.5±79.8	239.5±59.3	0.745
G6PD (U/g Hb)	6.9±2.4	7.6±2.0	7.8±3.4	8.1±2.4	0.423

The results are shown as mean±SD.

Table IV. Prognostic Factors for NPC Recurrence Examined by Univariate Analysis

Parameter	Variable	Number	2-year recurrence-free rate	P value
Age	≤40	35	0.960	0.217
	>40	73	0.823	
T stage	T1	40	0.904	0.065
	T2	14	0.758	
	T3	28	0.950	
	T4	26	0.719	
N stage	N0	34	0.850	0.941
	N1	47	0.861	
	N2	16	0.875	
	N3	11	0.857	
G6PD (U/10^{12} RBC)	≤130	9	0.500	0.0004
	>130	99	0.903	
G6PD (U/g Hb)	≤4	11	0.600	0.0082
	>4	97	0.900	

Log-rank test was used for analysis.

Table V. Prognostic Factors for NPC Metastasis Examined by Univariate Analysis

Parameter	Variable	Number	2-year metastasis-free rate	P value
Age	≤40	35	0.844	0.679
	>40	73	0.827	
T stage	T1	40	0.881	0.361
	T2	14	0.909	
	T3	28	0.839	
	T4	26	0.688	
N stage	N0	34	0.874	0.310
	N1	47	0.816	
	N2	16	0.681	
	N3	11	0.910	
G6PD (U/10^{12} RBC)	≤130	9	0.762	0.616
	>130	99	0.832	
G6PD (U/g Hb)	≤4	11	0.808	0.880
	>4	97	0.828	

Log-rank test was used for analysis.

Table VI. Prognostic Factors for NPC Recurrence Examined by Multivariate Analysis

Parameter	Variable	Hazard ratio (HR)	95% CI of HR	P value
Age	≤40	1.00	reference	0.833
	>40	1.19	0.23–6.11	
T stage	T1	1.00	reference	0.324
	T2	1.97	0.46–8.46	
	T3	0.23	0.03–2.14	
	T4	1.44	0.29–7.19	
N stage	N0	1.00	reference	0.644
	N1	1.71	0.32–9.19	
	N2	0.54	0.08–2.59	
	N3	1.68	0.15–18.33	
G6PD (U/10^{12} RBC)	≤130	7.73	1.59–37.5	0.011
	>130	1.00	reference	

Cox's proportional hazard model was used for analysis.

Table VII. Prognostic Factors for NPC Metastasis Examined by Multivariate Analysis

Parameter	Variable	Hazard ratio (HR)	95% CI of HR	P value
Age	≤40	1.00	reference	0.455
	>40	0.64	0.19–2.08	
T stage	T1	1.00	reference	0.804
	T2	0.76	0.08–6.88	
	T3	1.57	0.38–6.49	
	T4	5.83	1.32–25.8	
N stage	N0	1.00	reference	0.111
	N1	3.98	0.86–18.38	
	N2	6.85	1.25–37.45	
	N3	0.96	0.10–9.34	
G6PD (U/10^{12} RBC)	≤130	1.81	0.27–12.02	0.538
	>130	1.00	reference	

Cox's proportional hazard model was used for analysis.

to cellular dysfunction and thus, promote cancer development. In the present study, we find that G6PD activity is significantly lower in NPC patients than in normal controls. This finding supports our hypothesis that, since the major role of G6PD is to generate NADPH to protect cells from oxidative insults, patients deficient in G6PD will be more susceptible to oxidative attack and prone to NPC development.

Aside from the direct effects of oxidative insults in patients with low G6PD activity, the increased oxidative stress may change viral (such as EBV) virulence or enhance viral replication in infected patients, leading to the promotion or recurrence of NPC. Recent observations of multiple pathogenetic interactions between ROS and viruses have indicated that such interactions may play important roles in the pathogenesis of many viruses (refs. 18, 19 for review). For example, in a cell culture system, H_2O_2 promotes the replication of human immunodeficiency virus (HIV), and antioxidants such as N-acetylcysteine have the opposite effect.[20] It is thought that oxidative stress enhances HIV replication through activating the binding of NF-κB transcription factor to the HIV virus. Another example is the change of viral virulence by alteration of the genomic sequence, through infecting into different redox states of the host cells. Another RNA

enterovirus, which does not cause myocarditis in selenium-sufficient mice, was shown to cause extensive cardiac pathology in mice consuming a diet deficient in selenium or vitamin E.[21] Although there is no report to date regarding the effects of oxidative stress on either the EBV replication rate or virulence, our hypothesis that low G6PD activity may render individuals more susceptible to EBV-induced tumorigenesis and recurrence of NPC deserves serious consideration. It would be important to correlate G6PD activity and EBV titer in a subsequent study in order to help to delineate the effect of oxidative stress on EBV infectivity.

Apart from the etiologic significance of the relation between G6PD-deficiency and NPC, this finding has important therapeutic implications for NPC. Radiotherapy is the major modality of NPC treatment. The biological basis for radiotherapy is to generate ionizing free radicals and subsequently to induce cellular stress responses leading to cell death.[22] Since the major biochemical function of G6PD in human cells is to generate NADPH, the clinical relevance of low G6PD activity may be related to changes in redox status which would alter the effects of radiation therapy. In the present study, we have found that the tumor recurrence rate is higher in NPC patients with low G6PD activity, indicating that these patients may possess higher radiation resistance. One possible explanation is that NADPH is involved in the pathway of radiation therapy. Although NADPH is a powerful reducing agent, it is also involved in generating radicals in biological systems. For example, the generation of NO by nitric oxide synthase (NOS) and other ROS by NADPH oxidase in human cells is NADPH-dependent.[23, 24] We previously found that in G6PD-deficient granulocytes the production

of NO, superoxide and H_2O_2 is impaired due to the decrease of NADPH.[25] In this regard, patients with low G6PD activity may have increased radiation resistance due to impaired production of free radicals which are responsible for the killing of cancer cells. As regards NPC prognostic factors, clinical staging did not predict tumor control in this study. This may be due to the short follow-up time for the patients. In this circumstance, G6PD already shows significant prognostic implications, indicating an important role of this enzyme in the early determination of NPC prognosis. For those patients with low level of G6PD, application of concomitant chemoradiotherapy or altered fractionation radiotherapy may improve tumor control.[26, 27]

Although the factors modulating radiation response have not been clearly defined, the intrinsic radiosensitivity of the patients is the most important factor in tumor control. For this reason, it may be worthwhile to optimize the radiation dose for the treatment of NPC patients with low G6PD activity in order to obtain maximal therapeutic benefit and to minimize complications. The mechanism underlying radiation resistance in NPC patients with low G6PD activity, as well as the optimal therapeutic radiation dose, should be further investigated.

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