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ESTABLISHMENT OF HIGH RISK POPULATION AND PRECANCEROUS LESION OF NASOPHARYNGEAL CARCINOMA (NPC)

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A prospective study was done by the examination of nasopharyngoscope, reaction of EB virus's antigens and antibodies, nasopharyngofibroscope, pathological and EB virus's DNA, EBERs, etc. of about 100000 persons in high risk area of NPC in Guangdong Province, China from 1986 to 1995. If any one of the following four conditions is present in some persons, i.e., (1) EBV VCA/IgA titer>1:80, (2) EBV EDAb>60%, (3) Dual or triple positiveness in VCA/IgA, EA/IgA and EDAb, (4) Any one of VCA/IgA, EA/IgA and EDAb keeps high titer or going up, they should be regarded as in precancerosis of NPC. The moderate or severe heteroplasia and heterometaplasia of nasopharyngeal mucosa are the precancerous lesions of NPC. Some individual who is in precancerosis or with precancerous lesion should be regarded as the high risk population of NPC. The results are of important scientific basis for screening and second degree prevention of NPC.

Key words: Nasopharyngeal carcinoma, EB virus, High risk population, Precancerous lesion.

Guangdong province is the highest risk area of NPC in China and even in the whole world, the mortality rate was $22.06/10^5$ for male, $10.85/10^5$ for female (WSMR)[1] in Sihui, people's life and labour were affected seriously in this area. How to find out the high risk persons and early patients with cancer and to interrupt the course and treat them are the important problems and should be solved urgently to oncological workers.

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MATERIALS AND METHODS

Region and Subject

A prospective study was done to a total of 98, 180 healthy persons aged 30–59 in Zhongshan, Sihui and Guangzhou, Guangdong Province from 1986 to 1995.

Items Observed

According to the screening program, items observed for the population were as follows: (1) nasopharyngeal examination of nasopharyngoscope, (2) examination of EBV VCA/IgA (shell antigen's IgA antibody), EA/IgA (early antigen's IgA antibody) and EDAb (neutralization ratio of DNA enzyme), (3) nasopharyngeal examination of nasopharyngofibroscope, biopsy and pathology, and (4) nasopharyngeal tissue examination of EBV DNA and EBERs.

All of the above examinations were done by Cancer Hospital and Cancer Institute, Sun Yat-Sen University of Medical Sciences.

All of the experimental data were analysed by SPSS 6.0 (statistical package for the social science 6.0) in IBM PC 586 computer.

RESULTS

Detection Rate of NPC

Fifty-four patients with NPC were detected in the first screening in three areas, the detection rate was $55.00/10^5$. After that, 7255 persons who were>1:5 of VCA/IgA titer and 2466 who were <1:5 of VCA/IgA titer were examined in pairs in 1:2 and with the same sex, aged ± 2 years for persons who were >1:40 of VCA/IgA titer, all people above were survived regularly until the end of 1994, 91 new cases of NPC were detected. Totally 145 patients with NPC were diagnosed pathologically in the population detection rate of NPC was $117.01/10^5$ persons per year.

Detection Rate of NPC with Various Reactions of EBV Antigen and Antibody

Detection Rate of NPC with Different Titers of VCA/IgA

NPC could be detected in positive (>1:5) and negative (<1:5) persons of VCA/IgA, but there was significant difference between them an between various titer positive populations (Table 1).

Detection Rate of NPC with Different Titers of EA/IgA

NPC could be detected in positive (>1:5) and negative (<1:5) persons of EA/IgA, but there was significant difference between them (Table 2).

Detection Rate of NPC with Different EDAb

The anti-enzyme rate (AER) was <30% in normal individuals.[2] The detection rate of NPC with different EDAb were shown in Table 3.

Detection Rate of NPC with Dual or Triple Positiveness in VCA/IgA, EA/IgA and EDAb

The detection rate of NPC was high in different combination of positive groups (Table 4).

Detection Rate of NPC with Any One of VCA/IgA, EA/IgA and EDAb Keeps Going Up or the Titer Keeps High

It was $583.09/10^5$ persons per year (x^2=896.14 P<0.001).

1. The detection rate of NPC for different titers of EBV VCA/IgA

	Titer of EBV VCA/IgA			
	<1:5[1]	>1:5[2]	>1:40[3]	>1:80[4]
No. of NPC	3	142	93	85
No. of persons observed	100646	56104	14418	4738
Detection rate (/100000 persons per year)	2.98	253.1	645.02	1794.0

1:2 x^2=490.95 P<0.0001; 2:3 x^2=17.95 P<0.0001; 3:4 x^2=17.44 P<0.0001

Table 2. The detection rate of NPC for different titers of EBV EA/IgA

	Titer of EBV EA/IgA		
	<1:5[1]	>1:5[2]	>1:10[3]
No. of NPC	74	71	44
No. of persons observed	50161	1923	358
Detection rate (/100000 persons per year)	147.52	3692.14	12307.05

1:2 x^2=293.86 P<0.001; 2:3 x^2=15.27 P=0.0001

Table 3. The detection rate of NPC for different AER of EBV EDAb

	Percentage of EBV EDAb		
	<30[1]	>30[2]	>60[3]
No. of NPC	16	44	19
No. of persons observed	26338	2461	551
Detection rate (/100000 persons per year)	60.74	1787.9	3448.27

1:2 x^2=111.76 P<0.0001; 2:3 x^2=1.4888 P=0.2224;

Table 4. The detection rate of NPC for different combination of positive groups

	VCA/IgA, EA/IgA[1] EDAb	VCA/IgA[2] EA/IgA	VCA/IgA[3] EDAb	EA/IgA[4] EDAb
No. of NPC	16	71	49	16
No. of persons observed	219	2073	1179	219
Detection rate (/100000 persons per year)	7305.9	3424.9	4156.1	7305.9

1:2 x^2=2.09 P=0.1482; 2:3 x^2=0.415 P=0.5195; 3:4 x^2=0.9199 P=0.3375

Sensitivity and Specificity of VCA/IgA, EA/IgA and EDAb for Detection of NPC

The sensitivity and specificity were calculated to evaluate the value of NPC diagnosis (Table 5).

Detection Rate of NPC with Nasopharyngeal Mucosa Heterotypic Lesion

Ninety-eight cases with nasopharyngeal mucosa minimal heteroplasia/heterometaplasia and 112 cases with moderate or severe heteroplasia/heterometaplasis were detected in this series, the detection rate of NPC was 1106.00/10^5 persons per year in moderate or severe heteroplasia/heterometaplasia, 5 of 6 cases with severe heteroplasia/heterometaplasia occurred canceration or infiltrating cancer.

Determination of EBV DNA in Nasopharyngeal Tissue

The amplifying target sequence for Polymerase Chain Reaction (PCR) was chosen within the Internal Repeat 1 (IR1) of EBV genome to detect the nasopharyngeal tissue. The results were seen in Table 6.

The results showed that EBV DNA could be seen not only in poor-differentiated but also in well-differentiated carcinoma of nasopharynx.

Determination of EBV EBERs in Nasopharyngeal Tissue

EBERs specific ssRNA probes transcribed from the recombinant plasmid pSP65 in which the genes of EBERs were inserted, were used to detect the expression of EBERs in nasopharyngeal biopsies (Table 7).

The results showed that EBV EBERs could be detected not only in carcinoma of nasopharynx but also in a part of nasopharyngeal mucosa heteroplasia/heterometaplasia.

Table 5. Sensitivity and specificity of reaction of EBV antigens and antibodies on NPC diagnosis

Sensitivity	Specificity	+PV	−PV	Accuracy	FNR	FPR	PLR	NLR
75.47	98.31	4.17	99.89	98.29	0.02	95.83	44.66	0.25

+PV=Positive Predictive Value; − PV=Negative Predictive Value; FNR= False Negative Reaction rate;
FPR=False Positive Reaction Rate; PLR=Positive Likelihood Rate; NLR=Negative Likelihood Rate

Table 6. Detection of EBV DNA extracted from paraffin blocks by PCR

Diagnosis of pathology	Biopsied tissues	No. containing EBV DNA/No. of samples
Well-differentiated carcinoma of NP	Nasopharynx	5/5
Poor-differentiated carcinoma of NP	Nasopharynx	4/4
Chronic inflammatory	Nasopharynx	0/5
Normal tissue	Nasopharynx	0/4
Normal human embryonic epithelial cell	Nasopharynx	0/1

Table 7. Expression of EBV EBERs in nasopharyngeal tissue

Diagnosis of pathology	Biopsied tissues	No. of positive/No. of samples
Well-differentiated carcinoma of NP	Nasopharynx	1/2
Poor-differentiated carcinoma of NP	Nasopharynx	22/22
Metastatic carcinoma from NPC	Neck lymphatic gland	1/1
Heteroplasia/heterometaplasia	Nasopharynx	3/8
Simple hyperplasia/metaplasia	Nasopharynx	0/10
Normal tissue	Nasopharynx	0/20

DISCUSSION

Establishment of High Risk Population and Precancerous Lesion of NPC

According to our researches before, there is a long precancerous period including precancerosis and precancerous lesion, precancerosis if a kind of functional change in human body but precancerous lesion is a kind of pathological change in ansopharyngeal tissue. NPC is associated with EBV closely.[3] Epithelial cell could be induced as canceration by whole EBV.[4] There are great differences of various reactions of antigen and antibody not only between patient and normal person[1] but antibodies appearance is also earlier than diagnosis pathologically about 4 to 46 months; detection of NPC in positive population is 40 times higher than in negative;[3] application of EBV antibodies for early diagnosis of NPC has been evaluated before by us.[5] Therefore, persons who are in precancerosis or precancerous lesion should be regarded as high risk population of NPC.

Any one of the following conditions should be regarded as precancerosis: (1) Titer of EBV VCA/IgA is >1:80 (detection rate of NPC 1794.00/10^5 persons per year, sensitivity 50.9%, specificity 95.2%), (2) AER of EDAb is >60% (detection rate of NPC 3448.27/10^5 persons per year, sensitivity 61.9%, specificity 96.4%), (3) Dual or triple positiveness in VCA/IgA (>1:5), EA/IgA (>1:5) and EDAb (>30%) (detection rate of NPC 3424.9–7305.9/10^5 persons per year), (4) Any one of VCA/IgA, EA/IgA and EDAb keeps high titer or going up (detection rate of NPC 583.09/10^5).

Examination of nasopharyngofibroscope and pathology should be given to them to find out early patient with carcinoma or precancerous lesion .

Precancerous Lesion of NPC

Nasopharyngeal mucosa heteroplasia/heterometaplasia had been regarded as precancerous lesion by pathologists and experimental oncologists.[6,7] In the same way, nasopharyngeal mucosa moderate or severe heteroplasia/heterometaplasia should also be regarded as the precancerous lesion of NPC in the results of our prospective study (detection rate of NPC 1106.00/10^5).

Evaluation of Risk of Canceration in Precancerous Lesion

Although abnormal nasopharyngeal mucosa is associated with NPC[8] and nasopharyngeal mucosa heteroplasia/heterometaplasia had been regarded as precancerous lesion by pathologists, but not all of

these cases develop to canceration, reversible change of precancerous lesion has been found, and even reversed to normal mucosa.[7] How to evaluate the risk of canceration? According to our research, the following condition, i.e., (1) EBV DNA detected in precancerous lesion, or (2) EBV EBERs expressed positively, can be regarded as the high risk index of canceration.[9–11] But those cases are still not enough, more precancerous lesion cases should be investigated further.

Etiology of NPC is still not clear, it is difficult to perform the first degree prevention. Therefore, the establishment of high risk population and precancerous lesion of NPC is useful to provide scientific base for second degree prevention-early detection, early diagnosis and early treatment .

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