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(54) **CIRCULATING EPSTEIN-BARR VIRUS DNA IN THE SERUM OR PLASMA OF PATIENTS FOR THE PREDICTION AND DETECTION OF EPSTEIN-BARR VIRUS ASSOCIATED CANCERS APART FROM HEAD, NECK AND LYMPHOID MALIGNANCIES**

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(57) **ABSTRACT**

The present invention features methods for diagnosing, detecting, monitoring and determining the prognosis of Epstein Barr virus associated cancers apart from head, neck and lymphoid malignancies in a patient by detecting or measuring EBV DNA present in the serum or plasma of the patient. The present invention also features diagnostic kits comprising suitable reagents for detecting EBV DNA in the serum or plasma of a patient.

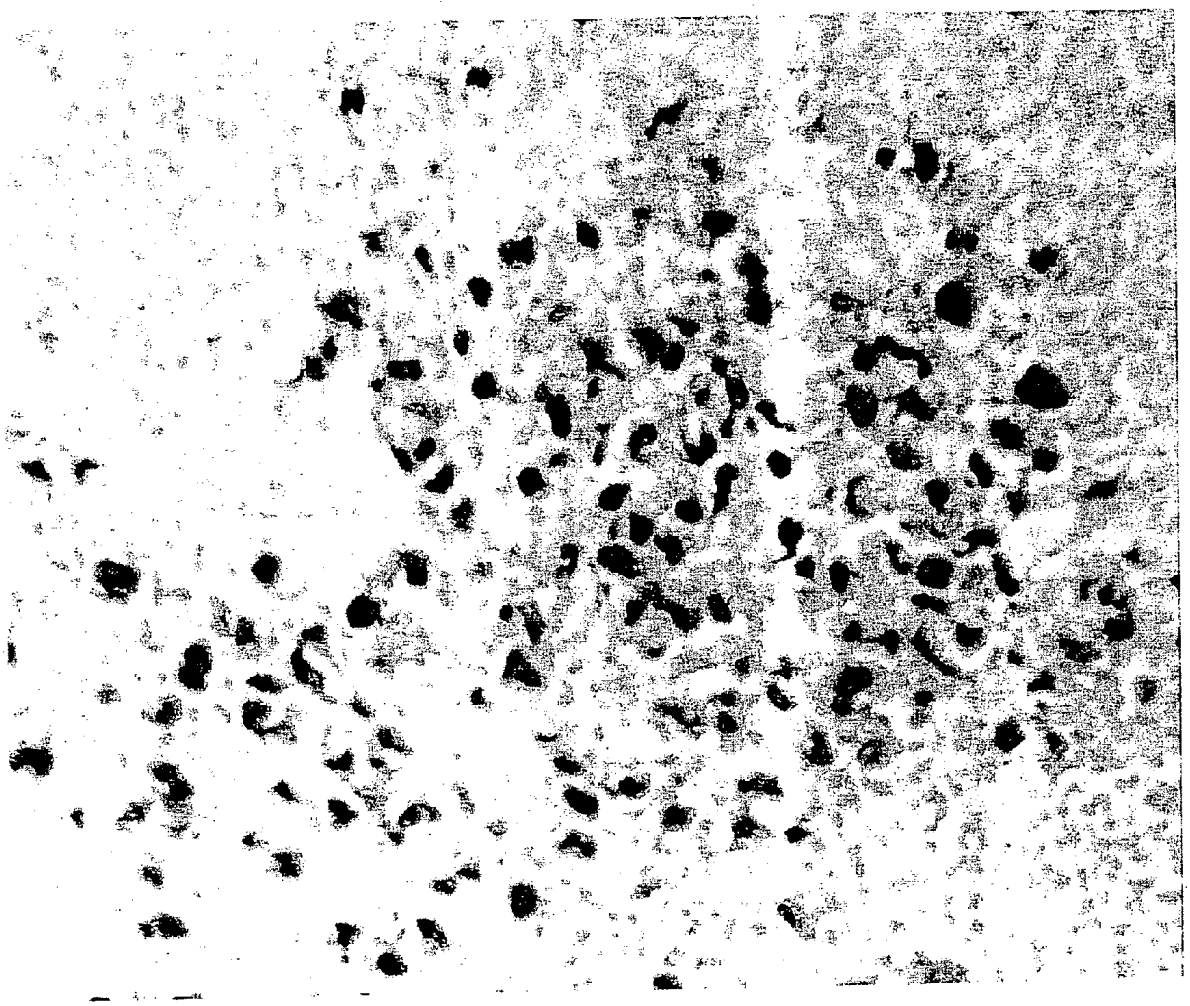


Figure 1A

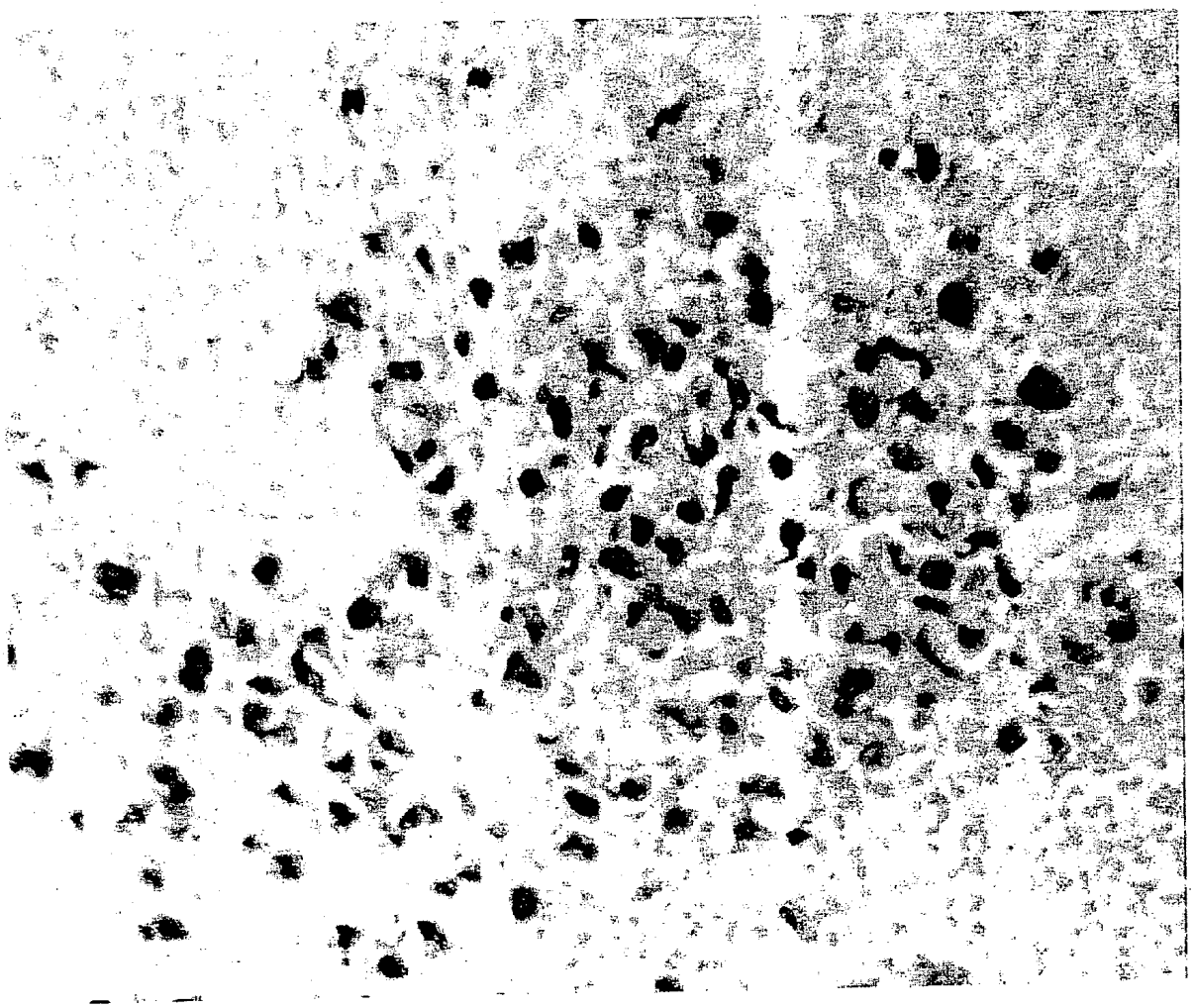
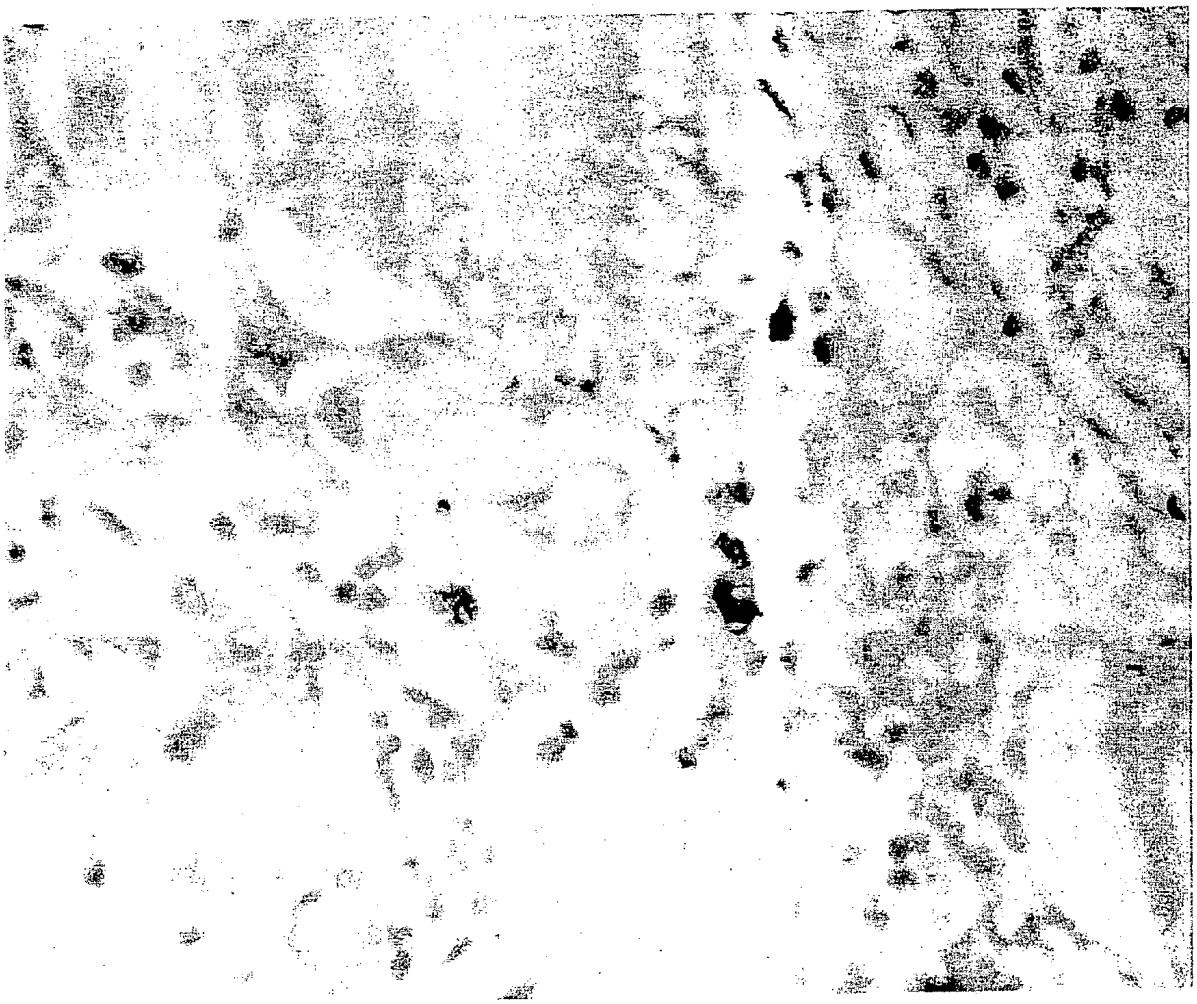
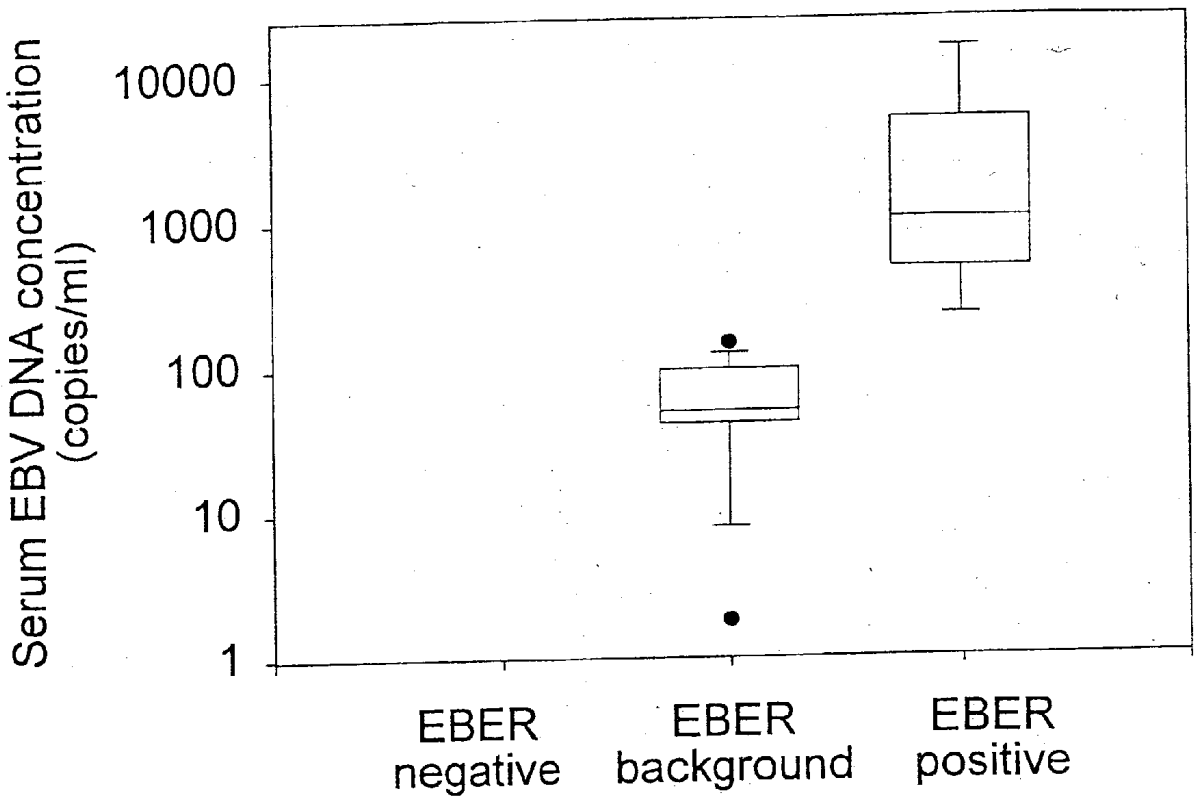


Figure 1B

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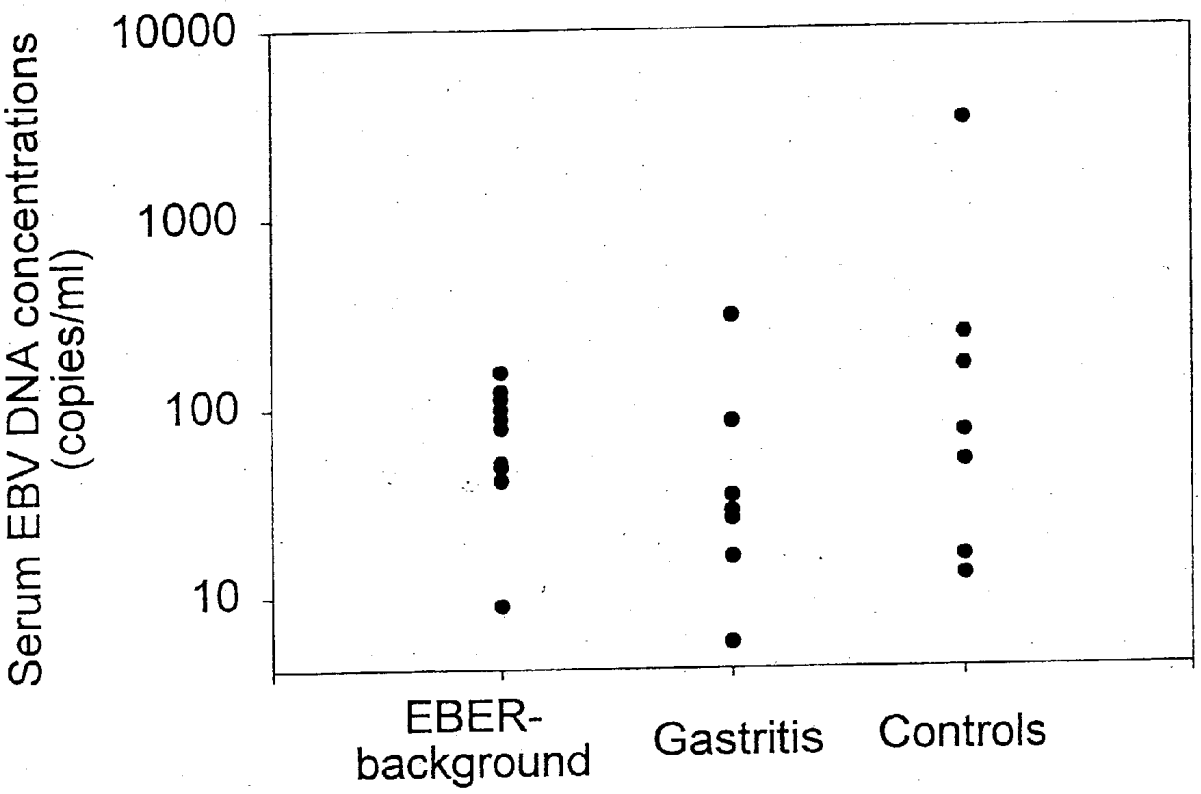
10000
1000
100
10
1
EBER negative
EBER background
EBER positive

Figure 2

Figure 3


Serum EBV DNA concentrations (copies/ml)
10000
1000
100
10
EBER-background
Gastritis
Controls

CIRCULATING EPSTEIN-BARR VIRUS DNA IN THE SERUM OR PLASMA OF PATIENTS FOR THE PREDICTION AND DETECTION OF EPSTEIN-BARR VIRUS ASSOCIATED CANCERS APART FROM HEAD, NECK AND LYMPHOID MALIGNANCIES

FIELD OF THE INVENTION

[0001] This invention relates to the discovery that Epstein Barr virus may be found in the cell free fluid of a patient's blood and when such virus is found, the patients may be suffering from Epstein Barr virus associated cancers apart from head, neck and lymphoid malignancies.

BACKGROUND OF THE INVENTION

[0002] It is known that tumour-derived DNA can be released by cancer cells of a variety of tumours (Anker et al., Cancer Metastasis Rev. 18: 65-73 (1999)). Examples include oncogene mutations from pancreatic carcinoma (Anker et al., Gastroenterology. 112: 4-1120 (1997)), microsatellite alterations in lung cancer (Chen et al., Nature Medicine. 2: 3-1035 (1996)) and epigenetic changes from liver cancer (Wong et al., Cancer Res. 59: 3 (1999)). In addition, virus DNA has been found in the circulation of a number of cancers known to be associated with virus infection. Examples include Epstein-Barr virus (EBV) DNA from nasopharyngeal cancer (Mutirangura et al., Clin Cancer Res. 4: 665-9 (1998); Lo et al., Cancer Res. 59: 1188-91 (1999)) and certain lymphomas (Lei et al., Br J Haematol. 111: 239-46 (2000); Gallagher et al., Int J Cancer. 84: 442-8 (1999); Drouet et al., J Med Virol. 57: 383-9 (1999)), and human papillomavirus DNA from head and neck cancer (Capone et al., Clin Cancer Res. 6: 4171-5 (2000)).

[0003] Recently, much interest has been focused on the presence of tumor-derived DNA in the plasma and serum of cancer patients (Chen, X. Q. et al., Nat. Med., 2: 1033-1035 (1996); Nawroz, H. et al., Nat. Med., 2: 1035-1037 (1996)). For virally-associated cancers, cell-free tumor-associated viral DNA has been detected in the plasma and serum of patients (Mutirangura, A. et al., Cancer Res., 4: 665-669 (1998); Lo, Y. M. D. et al., Clin. Cancer Res. 59: 1188-1191 (1999); Capone, R. B. Clin. Cancer Res., 6: 4171-4175 (2000)). One important virus which has been associated with many types of malignancy is the Epstein-Barr virus (EBV) (Cohen, J. I. N. Engl. J. Med., 343: 481-492 (2000)). Epstein-Barr virus (EBV) is a human herpesvirus that infects the majority of the human population. EBV is commonly transmitted by saliva and established latent infection in B lymphocytes where it persists for the lifetime of the host. In this regard, circulating EBV DNA has been detected in the plasma and serum of patients with nasopharyngeal carcinoma (NPC) (Mutirangura, A. et al., Cancer Res., 4: 665-669 (1998); Lo, Y. M. D. et al., Clin. Cancer Res., 59: 1188-1191 (1999)) and certain lymphoid malignancies (Lei, K. I. et al., Br. J. Haematol., 111: 239-246 (2000); Drouet, E. et al., J. Med. Virol., 57: 383-389 (1999); Gallagher, A. et al., Int. J. Cancer, 84: 442-448 (1999)).

[0004] EBV infection has also been reported to be associated with a proportion of gastric carcinomas (Shibata, D. et al., Am. J. Pathol., 140: 769-774 (1992)). In Hong Kong, approximately 10% of gastric carcinoma cases have been found to be associated with EBV infection (Yuen, S. T. et al., Am. J. Surg. Pathol., 18: 1158-1163 (1994)).

[0005] The present invention provides methods for detecting EBV DNA in the sera of patients with EBV associated cancers apart from head, neck and lymphoid malignancies and correlating the amount of EBV DNA so detected into clinical diagnosis or prognosis.

BRIEF SUMMARY OF THE INVENTION

[0006] In a first aspect, the present invention features methods for diagnosing, detecting, monitoring and determining the prognosis of EBV associated cancers apart from head, neck and lymphoid malignancies in a patient. The methods feature detecting or determining the amount of Epstein Barr Virus DNA (EBV DNA) present in the serum or plasma of such patients. Accordingly, the present invention have broad applicability in clinical medicine especially latent EBV infection occur over 90% of some populations.

[0007] The methods according to the present invention are also applicable for diagnosing, detecting, monitoring and determining the prognosis of EBV associated cancers apart from head, neck and lymphoid malignancies, such as gastric, breast, liver, lung and colon cancers. These neoplasms have been associated with EBV infection.

[0008] The methods according to the present invention generally comprise the steps of (1) obtaining a blood sample from a patient, (2) extracting DNA from the blood sample, (3) measuring the amount of circulating EBV DNA present in the blood sample, and (4) comparing the amount of circulating EBV DNA present in the blood sample to a control.

[0009] Preferably, the blood sample is a non-cellular fluid sample. By non-cellular we mean that the sample is either blood sera where the cells are extracted by clotting and separation of the cells from the remaining fluid or by inhibiting clotting and centrifuging the fluid fraction (plasma). The EBV DNA is measured from the fluid fraction. When EBV is found in the fluid of a non-cellular sample, it is understood that the infection is active and infected cells releasing EBV.

[0010] In a second aspect, the present invention features diagnostic kits comprising suitable reagents for detecting EBV DNA in the serum or plasma of patients. The kits according to the present invention may further comprise one or more of a device for obtaining a blood sample from a patient, a means to separate the EBV DNA from the blood sample and a means to quantify the amount of EBV DNA present in the blood sample. Such kits are useful for diagnosing, detecting, monitoring and determining the prognosis for EBV associated cancers apart from head, neck and lymphoid malignancies.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] **FIG. 1A** depicts gastric adenocarcinoma with small EBV encoded RNA (EBER)-positive tumor cells. EBER in-situ hybridization, ×200 magnification. **FIG. 1B** depicts gastric adenocarcinoma with occasional EBER-positive tumor infiltrating lymphocytes. The tumor cells are negative. EBER in-situ hybridization, ×400 magnification.

[0012] **FIG. 2** illustrates the difference in the level of circulating EBV DNA amongst three patient groups. Serum EBV DNA was detected in every one of the EBER-positive cases (median serum EBV DNA concentration: 1063 copies/

mL; interquartile range: 485-5141 copies/mL). No serum EBV DNA was detected in any of the 32 negative cases. Thirteen out of the 14 cases (93%) demonstrating 'background' EBER positivity had detectable serum EBV DNA. These cases had an intermediate median serum EBV DNA concentration of 50 copies/mL (interquartile range: 42 to 98 copies/mL).

[0013] **FIG. 3** demonstrates a comparison between cases with detectable serum EBV DNA in the gastric carcinoma cases with 'background' EBER-positivity, gastritis cases and control subjects. The serum EBV DNA concentrations of these three groups are presented. There is no statistically significant difference in circulating EBV DNA levels amongst these three groups (Kruskal-Wallis test, p=0.296).

DETAILED DESCRIPTION OF THE INVENTION

[0014] The present invention features methods for diagnosing, detecting, monitoring and determining the prognosis of EBV associated cancers apart from head, neck and lymphoid malignancies in a patient. The methods feature detecting or determining the amount of EBV DNA present in the serum of these patients. The methods according to the present invention have broad applicability in clinical medicine.

[0015] Clinically, circulating EBV DNA is applicable in diagnosing and monitoring gastric carcinoma patients who have EBER-positive tumors, similar to what has been achieved for nasopharyngeal cancers (Lo, Y. M. D. et al., Clin. Cancer Res., 59: 1188-1191 (1999); Lo, Y. M. D. et al., Cancer Res., 59: 5452-5455 (1999)) and certain lymphomas (Lei, K. I. et al., Br. J. Haematol., 111: 239-246 (2000); Drouet, E. et al., J. Med. Virol., 57: 383-389 (1999); Gallagher, A. et al., Int. J. Cancer, 84: 442-448 (1999)). The recent demonstration of the prognostic significance of circulating EBV DNA in nasopharyngeal cancers (Lo, Y. M. D. et al., Cancer Res., 60: 6878-6881) suggests that EBV DNA measurement has prognostic importance for gastric carcinoma.

[0016] The methods according to the present invention are also applicable for detecting, monitoring and determining the prognosis of EBV associated cancers apart from head, neck and lymphoid malignancies where those cancers are associated with EBV. Some of these neoplasms have been shown previously to be associated with EBV infection (Bonnet et al., J Natl Cancer Inst. 91: 1376-81 (1999)), as have certain liver cancers (Sugawara et al., Virology. 256: 196-202 (1999)).

[0017] Any of the conventional DNA amplification or signal amplification methods may be used for detection of EBV DNA. In most instances, it is desirable to amplify the target sequence using any of several nucleic acid amplification procedures which are well known in the art. Specifically, nucleic acid amplification is the enzymatic synthesis of nucleic acid amplicons (copies) which contain a sequence that is complementary to a nucleic acid sequence being amplified. Examples of nucleic acid amplification procedures practiced in the art include the polymerase chain reaction (PCR), strand displacement amplification (SDA), ligase chain reaction (LCR), and transcription-associated amplification (TAA). Nucleic acid amplification is especially beneficial when the amount of target sequence present in a sample is very low. By amplifying the target sequences and detecting the amplicon synthesized, the sensitivity of an assay can be vastly improved, since fewer target sequences are needed at the beginning of the assay to better ensure detection of nucleic acid in the sample belonging to the organism or virus of interest.

[0018] Methods of nucleic acid amplification are thoroughly described in the literature. PCR amplification, for instance, is described by Mullis et al. in U.S. Pat. Nos. 4,683,195 Methods of nucleic acid amplification are thoroughly described in the literature. PCR amplification, for instance, is described by Mullis et al. in U.S. Pat. Nos. 4,683,195, 4,683,202 and 4,800,159, and in *Methods in Enzymology,* 155: 335-350 (1987). Examples of SDA can be found in Walker, *PCR Methods and Applications,* 3: 25-30 (1993), Walker et al. in *Nucleic Acids Res.,* 20: 1691-1996 (1992) and *Proc. Natl Acad. Sci.,* 89: 392-396 (1991). LCR is described in U.S. Pat. Nos. 5,427,930 and 5,686,272. And different TAA formats are provided in publications such as Burg et al. in U.S. Pat. Nos. 5,437,990; Kacian et al. in U.S. Pat. Nos. 5,399,491 and 5,554,516; and Gingeras et al. in International Application No. PCT/US87/01966 and International Publication No. WO 88/01302, and International Application No. PCT/US88/02108 and International Publication No. WO 88/10315.

[0019] Real-time quantitative PCR is a preferred means to monitor EBV DNA and is based on the continuous optical monitoring of the progress of a fluorogenic PCR reaction (Heide et al. *Genome Res.* 6: 986-694, 1996 and Lo et al. *Am J Hum. Genet.* 62: 768-775, 1998). In this system, in addition to the two amplification primers used in conventional PCR, a dual-labeled fluorogenic hybridization probe is also included (Livak, et al. *PCR Methods Appl.,* 4357-362, 1995). One fluorescent dye serves as a reporter (FAM), and its emission spectrum is quenched by a second fluorescent dye (TAMRA). During the extension phase of PCR, the 5' to 3' exonuclease activity of the Taq DNA ploymerase (9) cleaves the reporter from the probe, thus releasing it from the quencher and resulting in an increase in fluorescence emission at 518 nm.

[0020] The methods according to the present invention generally comprise the steps of (1) obtaining a blood sample from a patient, (2) extracting DNA from the blood sample, (3) measuring the amount of circulating EBV DNA present in the blood sample, and (4) comparing the amount of circulating EBV DNA present in the blood sample to a control. Preferably, the blood sample is centrifuged, a fluid fraction is obtained, and the EBV DNA is measured from the fluid fraction.

[0021] Those of skill in the art will understand that the DNA may be extracted from a blood sample by many means known in the art. One preferred means is using a QIAamp Blood Kit. Also, the amount of circulating EBV DNA may be measured using one of many known or novel protocols. A protocol comprising a real time PCR amplification system is particularly preferred. Standard procedures for comparing the levels of EBV DNA so detected to a control may easily be devised so as to statistically assess the significance of the values obtained.

[0022] The number of copies of EBV DNA may be measured over time and correlated to disease progression or regression. Thereby, the present invention provides a non-

invasive method that allows diagnosis and subsequent monitoring of gastric carcinoma, gastritis and certain other cancers.

[0023] In a second aspect, the present invention features diagnostic kits comprising suitable reagents for detecting EBV DNA in the serum or plasma of patients. The kits according to the present invention may further comprise one or more of a device for obtaining a blood sample from a patient, a means to separate the EBV DNA from the blood sample and a means to quantify the amount of EBV DNA present in the blood sample. Such kits are useful for diagnosing, detecting, monitoring and determining the prognosis of EBV associated cancers apart from head, neck and lymphoid malignancies.

[0024] All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

[0025] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

[0026] EXAPMPLES

[0027] The following examples are provided by way of illustration only and not by way of limitation. Those of skill will readily recognize a variety of noncritical parameters which could be changed or modified to yield essentially similar results.

Example 1

Materials and Methods

[0028] Fifty-one patients with gastric carcinoma were recruited with informed consent from the Prince of Wales Hospital, Hong Kong. Blood samples were taken before surgical resection of the tumor. Following operation, sections of the tumor were taken for in-situ hybridization analysis for EBER (small EBV encoded RNA). Blood samples were also taken from 30 individuals with gastritis, without evidence of cancer carcinoma, and 197 apparently healthy control subjects.

[0029] DNA Extraction from Plasma Samples. Peripheral blood (5 ml) can be collected from each subject into an EDTA tube for the isolation of plasma. Blood samples are centrifuged at 1600×g, and plasma carefully removed from the EDTA-containing tubes and transferred into plain polypropylene tubes. The samples are stored at −20° C. until further processing. DNA form plasma samples are extracted using a QIAamp Blood Kit (Qiagen, Hilden, Germany) using the blood and body fluid protocol as recommended by the manufacturer (2). Plasma samples (130-800 μl/column) are used for DNA extraction. The exact amount is documented for the calculation of the target DNA concentration. A final elution volumn of 50 μl is used from the extraction columns.

[0030] Circulating EBV DNA concentrations were measured using a real time quantitative PCR system towards the BamHI-W fragment region of the EBV genome (Lo, Y. M. D. et al., *Cancer Res.,* 59: 1188-1191 (1999)). The principals of real time quantitative PCR and reaction set-up procedures were as previously described (Lo, Y. M. D. et al., *Cancer Res.,* 59: 1188-1191 (1999)). Data were collected using an ABI Prism 7700 Sequence Detector and were analyzed using the Sequence Detection System software (version 1.6.3) developed by Applied Biosystems. Results were expressed as copies of EBV genomes per millititer of serum.

[0031] All serum DNA samples were also subjected to real time PCR analysis for the (beta-globin gene (Lo, Y. M. D. et al, *Cancer Res.,* 59: 1188-1191 (1999)), which gave a positive signal on all tested samples, thus demonstrating the quality of the extracted DNA. Multiple negative water blanks were included in every analysis.

[0032] More specifically, two real-time quantitative PCR systems have been developed for EBV DNA detection: (a) one toward the BamHI-W region; and (b) the other toward the EBNA-I region (Baer, et al *Nature,* 310: 207-211, 1984). The BamHI-W system consisted of the amplification primers (SEQ ID NO: 1) W-44F (5'-CCCAACACTCCACCACACC-3') and (SEQ ID NO: 2) W-119R (5'-TCTT AGGAGCTGTCCGAGGG-3') and the dual-labeled fluorescent probe (SEQ ID NO: 3) W-67T (5'-FAM)CACACACTACACACACCCAC-CCGTCTC(TAMRA)-3']. The EBNA-1 system consisted of the amplification primers (SEQ ID NO: 4) EBNA-1162F (5'-TCATCATCATCCGGGTCTCC-3') and (SEQ ID NO: 5) EBNA-1229R (5'-CCTACAGGGT-GGAAAAATGGC-3') and the dual-labeled fluorescent probe (SEQ ID NO: 6) EBNA-1186T [5'-(FAM)CGCAGGCCCCCTCCAGGTA-GAA(TAMRA)-3']. The fluorescent probes contained a 3'-blocking phosphate group to prevent probe extension during PCR. Primer/probe combinations were designed using Primer Express software (Perkin-Elmer Corp., Foster City, Calif.). Sequence data for the EBV genome were obtained from the GenBank Sequence Database (accession number V01555). Real-time quantitative PCR for the β-globin gene consisted of primers and probe, as described previously in Lo, et al. Am J Hum Genet 62: 768-775, 1998), and was used as a control for the amplifiability of plasma DNA.

[0033] Fluorogenic PCR reactions are set up in a reaction volume of 50 μL using components (except for the fluorescent probes and amplification primers) supplied in a TaqMan PCR Core Reagent Kit (Perkin-Elmer Corp.). Fluorescent probes are custom-synthesized by Perkin-Elmer Applied Biosystems. PCR primers were synthesized by Life Technologies, Inc. (Gaithersburg, Md.). Each reaction contained 5 μl of 10× buffer A; 300 nM of each of the amplification primers; 25 nM (for the EBV probes) or 100 nM (for the β-globin probe) of the corresponding fluorescent probe; 4 MM $MgCl_2$; 200 μm each of dATP, dCTP, and dGTP; 400 μM dUTP; 1.25 units of AmpliTaq Gold; and 0.5 unit of AmpErase uracil N-glycosylase.

[0034] DNA amplifications are carried out in a 96-well reaction plate format in a Perkin-Elmer Applied Biosystems 7700 Sequence Detector. Each sample are analyzed in duplicate. Multiple negative water blanks were included in every analysis.

[0035] A calibration curve is run in parallel and in duplicate with each analysis, using DNA extracted from the EBV-positive cell line Namalwa (American Type Culture

Collection CRL-1432; See Klein et al., *Int J. Cancer,* 10: 44-57, 1972) as a standard. Namalwa is a diploid cell line that contains two integrated viral genomes/cell. A conversion factor of 6.6 pg of DNA/diploid cell was used for copy number calculation (Saiki et al., Science, 239: 487-491, 1988). Concentrations of circulating cell-free EBV DNA were expressed as copies of EBV genome/ml plasma.

[0036] An identical thermal profile was used for the EBV BamHI-W and EBNA-I PCR systems. Thermal cycling was initiated with a 2-min incubation at 50° C. for the uracil N-glycosylase to act, followed by an initial denaturation step of 10 min at 95° C., and then 40 cycles of 95° C. for 15 s and 56° C. for 1 min were carried out.

[0037] Amplification data collected by the 7700 Sequence Detector and stored in a Macintosh computer (Apple Computer, Cupertino, Calif.) is then analyzed using the Sequence Detection System software developed by Perkin-Elmer Applied Biosystems. The mean quantity of each duplicate is used for further concentration calculation. The plasma concentration of EBV DNA or the β-globin gene (expressed in copies/ml) is calculated using the following equation:

$$C = Q \times \frac{V_{DNA}}{V_{PCR}} \times \frac{1}{V_{ext}}$$

[0038] in which C represents the target concentration in plasma (copies/ml), Q represents the target quantity (copies) determined by a sequence detector in a PCR, V_{DNA} represents the total volume of DNA obtained after extraction (typically 50 μl/Qiagen extraction), VPCR represents the volume of DNA solution used for PCR (typically 5 μl, and V_{ext} represents the volume of plasma/serum extracted (typically 0.13-0.80 ml)).

[0039] The presence of EBV in tumor cells was assessed by in-situ hybridization on paraffin-embedded tissue sections using a fluorescein-conjugated oligonucleotide probe for EBER (Novocastra, U.K.) as previously described (Hui, P. K. et al., *Hum. Pathol.,* 25: 947-952 (1994)).

Results

[0040] A total of 51 gastric carcinoma patients were recruited. In this cohort, 5 gastric carcinomas were EBER-positive (**FIG. 1A**). In 14 cases, the tumor cells were EBER-negative, but there were occasional infiltrating lymphocytes which were EBER-positive (**FIG. 1B**). These 14 cases were classified as having 'background' positivity. **FIG. 2** illustrates the difference in the level of circulating EBV DNA amongst these three patient groups. Serum EBV DNA was detected in every one of the EBER-positive cases (median serum EBV DNA concentration: 1063 copies/mL; interquartile range: 485 to 5141 copies/mL). No serum EBV DNA was detected in any of the 32 negative cases (**FIG. 2**). Thirteen out of the 14 cases (93%) demonstrating 'background' EBER positivity had detectable serum EBV DNA. These cases had an intermediate median serum EBV DNA concentration of 50 copies/mL (interquartile range: 42 to 98 copies/mL). The difference between these three groups is statistically significant ($p<0.001$, Kruskal-Wallis test). Pairwise multiple comparison analysis indicates significant difference between the EBER-positive and EBER-negative groups ($p<0.05$, Dunn's method) and between the EBER-background and EBER-negative groups ($p<0.05$, Dunn's method).

[0041] EBV DNA was detectable in the serum of 7 of the 30 gastritis samples (23%) and 7 of the 197 healthy controls (3.6%). The proportions of serum EBV DNA positive cases between these groups are significantly different (chi-square test, $p=0.028$). Even in the cases with detectable circulating EBV DNA, the actual srum EBV DNA concentrations were generally lower than those in the EBER-positive gastric carcinoma cases.

[0042] A comparison was made for the cases with detectable serum EBV DNA in the gastric carcinoma cases with 'background' EBER-positivity, gastritis cases and control subjects. The serum EBV DNA concentrations of these three groups are plotted in **FIG. 3**. There is no statistically significant difference in circulating EBV DNA levels amongst these three groups (Kruskal-Wallis test, $p=0.296$).

Discussion

[0043] These data demonstrate that cell-free EBV DNA can be detected in serum samples obtained from a proportion of gastric carcinoma patients. Since gastric carcinoma is not classified as a lymphoma or lymphocyte associated cancer like nasopharyngeal carcinoma or related head and neck cancers, this is the first time that cell-free EBV DNA is shown effective for the detection and diagnosis of EBV associated cancers apart from head, neck and lymphoid malignancies. In addition, these data demonstrate an interesting correlation between the detectability of serum EBV DNA and tumoral EBER status. Thus, EBER-positive gastric carcinoma cases were associated with high levels of serum EBV DNA; gastric carcinoma cases with 'background' EBER-positivity were associated with intermediate levels; and no serum EBV DNA was seen in EBER-negative cases. This observation lends further demonstrate that plasma and serum represent noninvasive sources of materials for monitoring cancer (Anker, P. et al., *Cancer Metastasis Rev.,* 18: 65-73 (1999)).

[0044] Clinically, circulating EBV DNA may have application in the diagnosis and monitoring in the proportion of gastric carcinoma patients who have EBER-positive tumors, similar to what has been achieved for NPC (Lo, Y. M. D. et al., *Cancer Res.,* 59: 1188-1191 (1999); Lo, Y. M. D. et al., *Cancer Res.,* 59: 5452-5455 (1999)) and certain lymphomas (Lei, K. I. et al., *Br. J Haematol.,* 111: 239-246 (2000); Drouet, E, et al., *J. Med. Virol.* 57: 383-389 (1999); Gallagher, A. et al., *Int. J. Cancer,* 84: 442-448 (1999)). Recently, the value of circulating EBV DNA in nasopharyngeal cancer prognosis has been demonstrated. The present data (Lo, Y. M. D. et al., *Cancer Res.,* 60: 6878-6881) indicate that EBV DNA measurement also has prognostic importance for gastric carcinoma.

[0045] The detection of circulating EBV DNA in gastric carcinomas demonstrating 'background' EBER-positivity is interesting. The EBER-positive lymphocytes infiltrating the tumor tissues may be the origin of the low levels of serum EBV DNA that are detectable in these cases. If this is correct then further work may elucidate the mechanism of EBV liberation by these EBER-positive lymphocytes. Mechanisms include active release of DNA (Rogers, J. C. et al.,

Proc. Natl. Acad. Sci. USA., 69: 1685-1689 (1972)) and activation of lytic EBV infection in a proportion of these cells.

[**0046**] The long-term significance of the presence of low levels of circulating EBV DNA in the blood of apparently healthy individuals remains to be elucidated. Importantly, future studies should address the possibility that these individuals might be at increased risk of developing EBV-associated diseases. This issue would be of tremendous public health and biological importance.

[**0047**] The present data also suggest that circulating EBV DNA may be useful in many other cancer types that are associated with EBV. Examples of such cancers include breast cancer (Bonnet, M. et al., *J. Natl. Cancer Inst.,* 91:-1376-1381 (1999)) and hepatocellular carcinoma (Sugawara, Y. et al., *Virology,* 256: 196-202 (1999)). As the association between some tumor types and EBV is still controversial, the possible detection of EBV DNA in the plasma of patients with such tumors may contribute towards resolving these issues.

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What is claimed is:

1. A method of determining the increased probability of a patient with increased Epstein Barr virus DNA in blood for the prognosis and diagnosis of Epstein Barr virus associated cancers apart from head, neck and lymphoid malignancies by:

(a) obtaining a sample of non-cellular blood-derived fluid from the patient; and

(b) assaying the fluid for the presence or absence of Epstein Barr virus where the presence of the virus is an indication of increased probability of the patient to have Epstein Barr virus associated cancers apart from head, neck and lymphoid malignancies.

2. A method of claim 1 where the patient can be diagnosed with Epstein Barr virus associated cancers apart from head, neck and lymphoid malignancies and the cancer cells are free of EBV nucleic acid.

3. A method of claim 1 where the patient can be diagnosed with Epstein Barr virus associated cancers apart from head, neck and lymphoid malignancies and the cancer cells contain EBV nucleic acid.

4. The method of claim 1 comprising the steps of:

(1) obtaining a blood sample from a patient;

(2) obtaining a fluid fraction from the blood sample;

(3) extracting DNA from the fluid fraction; and

(4) measuring the amount of circulating EBV DNA present in the fluid fraction.

5. The method of claim 4 further comprising the step of:

(5) comparing the amount of circulating EBV DNA present in the fluid fraction to a control.

6. A kit for determining the increased probability of a patient with increase EBV DNA in blood for the prognosis and diagnosis of Epstein Barr virus associated cancers apart from head, neck and lymphoid malignancies.

(a) nucleic acid for detecting Epstein Barr virus in the blood of patients suffering from; and

(b) instructions for use of the nucleic acid to determine the presence or absence of Epstein Barr virus and an explanation of the increased probability of the patient suffering from Epstein Barr virus associated cancers apart from head, neck and lymphoid malignancies.

7. A diagnostic kit for detecting EBV DNA in the serum or plasma of a patient comprising reagents suitable for detecting EBV DNA in the serum or plasma.

8. A diagnostic kit according to claim 6 comprising a device for obtaining a blood sample from a patient.

9. A diagnostic kit according to claim 6 comprising a means to separate EBV DNA from a blood sample.

10. A diagnostic kit according to claim 6 comprising a means to quantify the amount of EBV DNA present in the blood sample.

* * * * *

专利名称(译)	在患者的血清或血浆中循环epstein-barr病毒DNA，用于预测和检测除头，颈和淋巴恶性肿瘤外的epstein-barr病毒相关癌症		
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发明人	LO, YUK MING DENNIS CHAN, WING YEE NG, KWOK WAI		
IPC分类号	G01N33/569 C12N15/09 C12Q1/68 C12Q1/6886 C12Q1/70 G01N33/48 G01N33/50 G01N33/53 G01N33/566		
CPC分类号	C12Q1/705 C12Q1/6886		
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外部链接	Espacenet USPTO		

摘要(译)

本发明的特征在于通过检测或测量患者血清或血浆中存在的EBV DNA来诊断，检测，监测和确定患者的头部，颈部和淋巴恶性肿瘤之外的Epstein Barr病毒相关癌症的预后的方法。本发明还涉及诊断试剂盒，其包含用于检测患者血清或血浆中的EBV DNA的合适试剂。