(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 3 009 839 A1

(12) EUROPEAN PATENT APPLICATION
published in accordance with Art. 153(4) EPC

(43) Date of publication:
20.04.2016 Bulletin 2016/16

(21) Application number: **14810344.3**

(22) Date of filing: **29.04.2014**

(51) Int Cl.:
G01N 33/53 *(2006.01)* ***G01N 21/76*** *(2006.01)*
G01N 30/72 *(2006.01)*

(86) International application number:
PCT/KR2014/003770

(87) International publication number:
WO 2014/200178 (18.12.2014 Gazette 2014/51)

(84) Designated Contracting States:
AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR
Designated Extension States:
BA ME

(30) Priority: **14.06.2013 KR 20130068324**
12.07.2013 KR 20130082024

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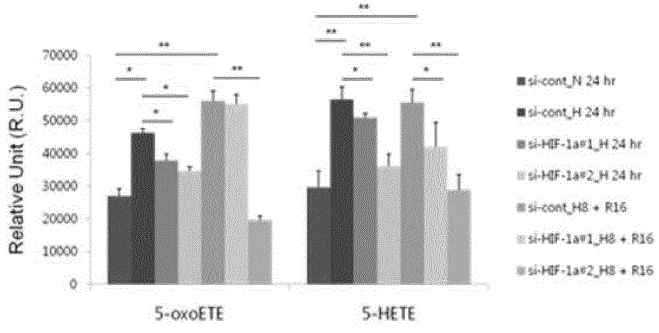
(54) METHOD FOR DETECTING HYPOXIA OR DIAGNOSING HYPOXIA-RELATED DISEASES

(57) The present invention provides a composition, kit, and method for detecting hypoxia or diagnosing hypoxia-related diseases, the composition containing a material for detecting arachidonic acid and a derivative thereof The composition, kit, and method according to the present invention can conveniently and promptly detect hypoxia through the detection of a biomarker in a biological sample, and thus can be useful in the prevention or early diagnose of diseases caused by hypoxia, the determination of the severity of diseases and therapeutic effects, tracking of diseases, or the like.

[FIG. 3]



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Description

BACKGROUND OF THE INVENTION

Field of the Invention

[0001] The present disclosure relates to a field of detecting hypoxia or diagnosing hypoxia-related diseases.

Description of the Related Art

[0002] Hypoxia is generally regarded as a pathological status in which oxygen required to sustain a human life is insufficiently supplied. It has been known that systemic hypoxia is as a fundamental cause of various diseases, such as, obstructive sleep apnea, asthma, chronic obstructive pulmonary disease (COPD, Lung fibrosis), pulmonary hypertension and pulmonary edema, pulmonary thromboembolism, cardiac failure, hypoxic ischemic encephalopathy, and perinatal asphyxia (Savransky et al. Am J Respir Crit Care Med. 2007; 175 (12):1290-7). Further, local hypoxia is a central pathophysiological factor of major detrimental diseases, such as, cerebrovascular diseases, cardiovascular diseases, and tumors. A living body may be frequently exposed to a hypoxia status due to various causes, such as, environmental changes (an alpine region, and the like) or respiratory diseases, but the homeostasis of oxygen is finely adjusted or controlled by various compensation mechanisms at an individual as a whole and tissue levels.

[0003] For such hypoxia-related diseases, determining the exact condition or extent of hypoxia under which a body as a whole or a specific organ has been exposed is a key to understand the cause of the diseases and diagnose them. However, up to now, diagnosis of hypoxia stays at the level of measuring oxygen saturation level in the blood at a particular moment. Optical oxygen saturation meters are widely used for non-invasive determination of oxygen concentration of the blood and measure oxygen saturation levels based on the difference in the absorbance resulting from the differential degree of oxygen binding to hemoglobin. More invasive way is an arterial blood gas analysis test in which the amount of oxygen, carbon dioxide and the like in the blood is measured using an arterial blood from a patient. The two methods described just estimate the oxygen level in the blood at a particular moment and do not provide any information regarding a hypoxic state a person has been exposed over a period of time. Further, in the conventional methods, the meter needs to be attached to the patient or invasive process such as arteriopuncture is often inevitable. Accordingly, there are needs to develop an improved assessment method of chronic hypoxia, which is crucial for accurate understanding of the pathological physiology of hypoxia-related diseases, and also for diagnosis, monitor and/or prevention of hypoxia-related diseases enabling correlation analysis between the severity of the disease and the extent or degree of exposure to a hypoxia condition.

[0004] It has been known that a hypoxia inducible factor (HIF) known for inducing hypoxia is a transcription factor regulating about 60 genes required for cells to adapt in a hypoxic condition and HIF target proteins are involved in biological processes such as angiogenesis and angiectasis, energy production, cell proliferation and survival, or cell migration, and the like (Brahimi-Horn MC, et al. J Mol Med. 2007;85(12):1301-7; Wykoff et al. Cancer Res. 2000;60(24):7075-83; Park JW et al. J Pharmacol Sci. 2004 ;94(3):221-32; and Gort et al. Curr Mol Med. 2008 ;8(1):60-7).

[0005] U.S. Patent Publication No. 2004-0265926 relates to bodily fluid markers of tissue hypoxia and discloses an oxygen related protein 150 (ORP 150) which is a marker capable of detecting hypoxia as one clinical symptom of a heart disease.

[0006] It is presumed that the metabolites cells produce and secrete during a hypoxic adaptation process may be significantly different in amounts and types from those secreted under a regular oxygen environment (Majmundar, et al. Mol Cell. 2010;40 (2):294-309), but there are no reports of such metabolites yet, and discovery of metabolite markers in relation to hypoxia is required.

SUMMARY OF THE INVENTION

[0007] In order to solve the conventional problems, the present disclosure is to provide biomarkers capable of detecting hypoxia and a method of detecting or diagnosing hypoxia-related diseases using the same.

[0008] In one aspect, the present disclosure provides a composition including a substance for detecting an arachidonic acid or a derivative thereof for detecting hypoxia or diagnosing hypoxia-related diseases, or a method for diagnosing hypoxia or hypoxia-related diseases using the same.

[0009] In an exemplary embodiment, the derivative of an arachidonic acid included in the present disclosure is a derivative produced in a metabolic pathway of 5-lipoxygease of the arachidonic acid, and particularly, the derivative of the arachidonic acid includes a 5-hydroperoxy eicosatetraenoic acid (5-HPETE), a 5-hydroxy-6,8,11,14-eicosatetraenoic acid (5-HETE), or a 5-oxo-6,8,11,14-eicosatetraenoic acid (5-oxoETE). However, the present disclosure is not limited thereto.

[0010] Detection substances which may be included in the composition of the present disclosure are not limited as long as they are capable of specifically recognizing an arachidonic acid or a derivative thereof. Examples thereof include receptors, ligands, substrates, antibodies, antibody fragments, antibody mimetics, aptamers, avidity multimers, or peptide mimetices, but the present disclosure is not limited thereto. The substance according to the present disclosure may be detected with various methods suitable for the substance, and for example, may be detected with an antibody analysis, chemiluminescent assay, or liquid chromatography-mass spectrometry assay, but the present disclosure is not limited thereto.

[0011] In an exemplary embodiment according to the present disclosure, the hypoxia-related diseases includes hypoxia-induced diseases, and for example, includes obstructive sleep apnea, asthma, chronic obstructive pulmonary disease (COPD, Lung fibrosis), pulmonary hypertension and pulmonary edema, pulmonary thromboembolism, cardiac failure, hypoxic ischemic encephalopathy, perinatal asphyxia, cerebrovascular diseases, cardiovascular diseases, or tumors, but the present disclosure is not limited thereto.

[0012] In another embodiment, the hypoxia according to the present disclosure includes acute systemic hypoxias, chronic systemic hypoxias, acute local hypoxia, and chronic local hypoxias.

[0013] The acute or chronic hypoxias disclosed in the present disclosure may be classified according to progression status of hypoxia. The acute hypoxias include asthma, pulmonary edema, pulmonary thromboembolism, airway obstruction, perinatal asphyxia, carbon monoxide poisoning, cerebrovascular and cardiovascular obstruction or bleeding, and the like, and the chronic hypoxias include obstructive sleep apnea, chronic obstructive pulmonary disease (COPD, Lung fibrosis), pulmonary hypertension, cardiac failure, anemia, hemoglobinopathy, tumors, and the like. However, the present disclosure is not limited thereto.

[0014] In the present disclosure, the systemic hypoxia may involve obstructive sleep apnea, asthma, chronic obstructive pulmonary disease (COPD, Lung fibrosis), pulmonary hypertension and pulmonary edema, pulmonary thromboembolism, cardiac failure, airway obstruction, pneumothorax, perinatal asphyxia, anemia, hemoglobinopathy, carbon monoxide poisoning, and cyanide poisoning, and the local hypoxias may involve cerebrovascular diseases, cardiovascular diseases, tumors, and ischemic tissue damages including hypoxic ischemic encephalopathy. However, the present disclosure is not limited thereto.

[0015] The diseases such as chronic or acute or systemic or local diseases described as above are not conclusive. In other words, a disease may show various combinations of several aspects of diseases, and thus various diseases disclosed in the present disclosure are not one particular clinical condition, but the changes in the body caused by hypoxia. For example, pulmonary edema is a disease in which parenchyma area is full of water, and thus, gas exchange between pulmonary alveoli and pulmonary vascular does not occur. When such a disease progresses rapidly, for example within 3 months or within several days to several weeks in other diseases, these diseases may be classified as being acute. However in cases where pulmonary edema is developed as a secondary disease accompanied by other underlying disease, the pulmonary edema may be classified as being chronic. In addition, in the light case of pulmonary edema in which only the peripheral region of a lung having edema is not supplied with oxygen and gas exchanges occur normally in other regions of a lung without edema to supply oxygen throughout the whole body, it may be called a local (only for partial lung) hypoxia. However, when gas exchange does not occur in most lungs due to a severe pulmonary edema, and thus, oxygen is not supplied throughout the whole body, systemic hypoxia may occur. For this reason, it is understood that hypoxias accompanied by various clinical diseases may include various aspects, such as, intermittent or persistent chronic hypoxias, acute hypoxias, hypoxic hypoxias, anemic hypoxias, congestive hypoxias, histotoxic hypoxias, or water-soluble hypoxias.

[0016] In another embodiment, the present disclosure provides a kit for detecting hypoxia or diagnosing hypoxia-related diseases, in which the kit includes the composition according to the present disclosure.

[0017] In another embodiment, the present disclosure provides a method for detecting a hypoxia marker for providing the information required for detecting hypoxia or diagnosing hypoxia-related diseases. In an exemplary embodiment, the method includes detecting an arachidonic acid or a derivative thereof, for example, a derivative produced in a metabolic pathway of 5-lipoxygenase of an arachidonic acid, such as, 5-HpETE, 5-HETE, or 5-oxoETE in the biological material isolated from a subject to be tested.

[0018] In the method according to the present disclosure, an arachidonic acid or a derivative thereof may be measured by any one or more methods of an antibody analysis, chemiluminescent assay, or liquid chromatography-mass spectrometry assay, but the present disclosure is not limited thereto.

[0019] In another embodiment, the present disclosure provides an arachidonic acid or a derivative thereof that is used for detecting hypoxia or diagnosing hypoxia-related diseases.

[0020] Arachidonic acids or a derivative thereof as a biomarker according to the present disclosure, or a composition or a kit comprising the same, or a method using the same may be used with or tested on various biological materials, in which the derivatives according to the present disclosure can be detected, for example, bodily fluids, such as, whole blood, plasma, serum, saliva, tear, sweat, and urine, hairs, cells, and tissues. However, the present disclosure is not limited thereto.

ADVANTAGEOUS EFFECTS

[0021] According to the present disclosure, simple but accurate detection of hypoxia is possible in a short period of time by detecting the metabolites of an arachidonic acid in a subject suspected of hypoxia-related disease or hypoxia. The present markers, compositions, kits and methods can be advantageously used to diagnose or early diagnose or detection of hypoxia, for example, sleep apnea syndrome, and to prevent various complications associated with hypoxia. Further using the present disclosure, monitoring the prognosis or determining of the therapeutic efficacies of the treatment may also be made.

BRIEF DESCRIPTION OF THE DRAWINGS

[0022]

FIG. 1 is a schematic diagram showing a 5-lipoxygenase (5-LO) pathway and metabolites of an arachidonic acid.
FIG. 2 is a graph showing the arachidonic acid-derived metabolites which are increased under a low oxygen condition in a monocyte-derived THP-1 cell line. N, normoxia (21% O_2); H, hypoxia (1% O_2); ReOxy, reoxygenation. *$P < 0.05$.
FIG. 3 is a result showing that a target metabolite is reduced when the hypoxia inducible factor is inhibited. N, normoxia (21% O_2); H, hypoxia (1% O_2); R, reoxygenation. *$P < 0.05$, **$P < 0.01$.
FIG. 4 is a result showing the decrease in the target metabolites when administering an inhibitor of a hypoxia inducible factor. N, normoxia (21% O_2); H, hypoxia (1% O_2); R, reoxygenation. *$P < 0.05$, **$P < 0.01$.
FIG. 5 shows the arachidonic acid-derived metabolites which are increased in a low oxygen condition in a human primary monocyte. N, normoxia (21% O_2); H, hypoxia (1% O_2); IH, intermittent hypoxia; R, reoxygenation. *$P < 0.05$, **$P < 0.01$.
FIG. 6 is a result showing that a target metabolite is reduced when a hypoxia inducible factor is inhibited in a human primary monocyte. N, normoxia (21% O_2); H, hypoxia (1% O_2); IH, intermittent hypoxia; R, reoxygenation; siH1, silencing HIF-lalpha. *$P < 0.05$, **$P < 0.01$.
FIG. 7 shows that the activity of glutathione peroxidase (GPX) transforming 5-HpETE into 5-HETE is increased in a hypoxic environment. N, normoxia (21% O_2); H, hypoxia (1% O_2); R, reoxygenation. * $P < 0.05$.
FIG. 8 is a result showing that the arachidonic acid-derived metabolites are increased in a first morning urine from an obstructive sleep apnea (OSA) patient.
FIG. 9 is a graph showing a correlation between the arachidonic acid-derived metabolites which are increased in urine of the OSA patient and minimum oxygen saturation.
FIG. 10 is a graph showing a correlation between the metabolites which are increased in urine of the OSA patient and an apnea-hypopnea index (AHI) .

[0023] In the present figures, all the statistical analyses were performed by using an IBM SPSS 18 (SPSS, Inc, Chicago, I11) statistical program and using a Mann-Whitney U test.

DETAILED DESCRIPTION OF THE EMBODIMENTS

[0024] The present disclosure is based on the discovery of biomarkers which are specifically present or expressed in a hypoxia condition and their use.

[0025] In one aspect, the disclosure relates to a use of the present biomarkers for determining or detecting hypoxia and disease related thereto. Particularly, in one embodiment, the present disclosure relates to a composition comprising a substance for detecting an arachidonic acid and a derivative thereof for detecting/diagnosing hypoxia or diagnosing hypoxia-related diseases and a method of detecting/diagnosing hypoxia or diagnosing hypoxia-related diseases using the present makers or compositions in a subject who is a hypoxic patient or a suspected of hypoxia.

[0026] In the present disclosure, the term "diagnosis" as used herein refers to determining the disease or disorder susceptibility of a subject, determining whether a subject has a specific disease or disorder, determining the prognosis (for example, determining the status of the disease or the response to the treatments) of a subject who has specific disease or disorder, or therametrics(for example, monitoring the status of a subject to provide the information on the efficacy of treatment).

[0027] In the present disclosure the term "detection" refers to determine the presence or absence or the extent of hypoxia and may be used interchangeably with diagnosis. For example, in the cases where a disease itself such as obstructive sleep apnea induces hypoxia, the detection of hypoxia is equivalent to diagnosis of the disease, and determining the extent of hypoxia may also be used to determine the severity of the disease. In other cases where a disease, such as for example pulmonary hypertension and hypoxic ischemic encephalopathy, is a result of hypoxia, the detection of hypoxic status of a body or a particular organ may be used for preventing and early diagnosing the corresponding

disease.

[0028] In the present disclosure, the term "biomarker or marker for diagnosing/detecting" refers to an agent that may discriminate a hypoxic sample or hypoxic status from a normal sample including samples undergone appropriate treatment and having normal characteristics. Also included are biomolecules such as lipids and glycolipids which are increased or decreased in a sample or a patient which suffers from the disease compared to normal samples. In the present disclosure, the biomarkers include an arachidonic acid and a derivative thereof, the amount of which is increased in hypoxia patient or samples.

[0029] The biomarker according to the present disclosure may be used alone or in a combination of two or more markers. Further, the present marker may be used in combination with existing diagnosis methods in the related art. Those skilled in the art may be able to select a combination of markers meeting the desired sensitivity and specificity in view of the analysis using biological materials from normal subject and patient as described in the present disclosure.s

[0030] In the present disclosure, the term hypoxia or hypoxic disease refers to a condition in which the body or a region of the body is deprived of adequate oxygen supply due to the reduced oxygen concentration in the blood or the increased amount of oxygen requirement by the tissue. The types include chronic intermittent or persistent hypoxia resulting from a reduced partial pressure of oxygen in the arterial blood, acute hypoxia, hypoxic hypoxia, anemic hypoxia, stagnant hypoxia, histotoxic hypoxia, and water-soluble hypoxia. In an exemplary embodiment of the present disclosure, the marker of the present disclosure may act as a cumulative marker reflecting the extent and the duration of hypoxia. In this respect, the present marker is particularly used for diagnosing persistent hypoxia or chronic intermittent hypoxia, particularly, persistent hypoxia.

[0031] In another aspect, the hypoxia of the present disclosure encompasses systemic or local hypoxia. The systemic hypoxia is accompanied by various diseases such as obstructive sleep apnea, asthma, chronic obstructive pulmonary disease (COPD, Lung fibrosis), pulmonary hypertension and pulmonary edema, pulmonary thromboembolism, cardiac failure, airway obstruction, pneumothorax, perinatal asphyxia, anemia, hemoglobinopathy, carbon monoxide poisoning, and cyanide poisoning without being limited thereto. Further, the local hypoxia is accompanied by a cerebrovascular disease, a cardiovascular disease, a cancer, and an ischemic tissue damage including hypoxic ischemic encephalopathy without being limited thereto.

[0032] In an exemplary embodiment of the present disclosure, the hypoxia is the one accompanied by obstructive sleep apnea. The hypoxia associated with obstructive sleep apnea is characterized by repeated cycles of reduction and restoration of oxygen saturation level in the blood in which the oxygen saturation level is reduced to for example 60% to 90% (to the extent that requires an oxygen mask) of the normal level due to the obstruction of an upper airway during sleep, followed by the restoration of the oxygen level to normal due to a strong respiratory drive (Somers VK et al. J Clin Invest 1995; 96: 1897-904). In certain cases, the oxygen saturation is lowered to a level less than 60% and in this case, possibility of sudden death is high.

[0033] In the present disclosure, the obstructive sleep apnea frequently causes respiratory arrest during sleep and may be divided into obstructive sleep apnea and central sleep apnea. Most of sleep apnea patients have anatomically characteristic phenotype in which the upper airway space is narrow. In sleep apnea syndrome patients, fat is frequently accumulated around airways due to obesity, or the oropharyngeal soft tissues such as tongue and tonsil are larger in size and thus the upper airway becomes narrow than in healthy controls. Most of the patients suffer from obstructive sleep apnea in which the upper airway is closed and thus the patients are exposed to the systemic hypoxia during sleep. Main symptoms include chronic intermittent hypoxia in which the oxygen saturation level is reduced to for example 60% to 90% (to the extent that requires an oxygen mask) of the normal level due to the obstruction of an upper airway during sleep, followed by the restoration of the oxygen level to normal due to a strong respiratory drive (Somers VK et al. J Clin Invest 1995; 96: 1897-904). Further, the obstructive sleep apnea acts as a cause for cardiovascular complications by secondarily exciting sympathetic nerves and thus promoting adipolysis in fat cells to increase the amount of free fatty acid in the blood (Hucking K. et al. J Clin Invest 2003;111:257-64), Hypoxic-oxygen restoration promotes the generation of reactive oxygen species (ROS) resulting in an oxidative tissue damage (Schulz R. et al. Am J Respir Crit Care Med 2000;162:566-70) and cytokines such as CRP, IL-6, and TNF-a cause inflammation (Ciftci TU et al. Cytokine 2004;28:87-91). In addition, adipokines such as leptin, adiponectin, and resistin are increased, adhesion molecules such as ICAM-1, VCAM-1, E-selectin, and L-selectin are increased, and endoplasmic reticulum stress is increased by accumulation of unfolded proteins (Tatsumi K et al.Chest 2005; 127: 716-21). As such, the obstructive sleep apnea may accompany cardiovascular complications such as hypertension, cardiac failure, cardiac arrhythmias, ischemic heart disease, stroke, and pulmonary hypertension, and it has been reported that mortality rate is increased statistically significantly in persons whose apnea index (the number of times of apnea per hour) is 20 or more (Shamsuzzaman AS et al. JAMA 2003; 290: 1906-14). Besides, secondary symptoms such as severe daytime lethargy and fatigue due to sleep fragmentation, retrograde memory loss that is a secondary symptom due to excessive sleepiness, decreased attention, decreased judgment, various personality changes (aggressive personality, irritability, anxiety, and depression), erectile dysfunction, and the like maybe developed (Simon S. et al. Chest. 2012 Dec;142(6):1645-51).

[0034] The biomarker of the present disclosure may be used for diagnosing or prognosis of hypoxia having various

symptoms and characteristics and for determining the severity of the disease. For the determination of the severity, the amount of metabolite measured can be correlated with the severity. For example, as shown in FIG. 5, more amounts of the metabolites are detected in the persistent hypoxia.

[0035] In the present disclosure, a biological sample or material refers to a substance or a mixture of the substances that contain or expected to contain one or more of the present biomarkers, and includes cells, tissues or bodily fluids from an organism, particularly human, for example, sweat, saliva, tears, whole blood, urine, plasma, and serum, or hair, but is not limited thereto. Further, the sample includes cells or tissues cultured in vitro as well as those derived directly from an organism. In an exemplary embodiment, urine, whole blood, plasma, and/or serum may be used. In another exemplary embodiment, particular fractionations or derivatives from the blood, cells, or tissues are included. When cells or tissues are used, lysates thereof may also be used.

[0036] In the present disclosure, the term detection or detecting refers to quantitative and/or qualitative analyses. The detection includes a determination of the presence and/or absence as well as the levels of the present markers. The present markers may be detected using the methods known in the art, and the person skilled in the art would be easily able to select appropriate methods for the detection.

[0037] The biomarker according to the present disclosure is arachidonic acid and derivatives thereof. The arachidonic acid (AA) is a polyunsaturated fatty acid which is mostly found in cell membranes and decomposed through two main metabolic paths, that is, one is the LO pathway where AA becomes a hydroxyl derivative by lipoxygenase (LO) and the other is the COX pathway where AA is converted to prostaglandin via cycloxygenase (COX).

[0038] In an exemplary embodiment of the present disclosure, the composition or the method of the present disclosure includes or use a material for detecting an AA metabolite generated particularly in the LO pathway. The LO pathway is schematically depicted in FIG. 1 and as shown in FIG. 1, 5-hydroperoxyeicosatetraeonic acid is an intermediate produced in a process in which an arachidonic acid is generated to leukotriene A4 and may be generated by arachidonate 5-lipoxygenase. 5-hydroxyeicosatetraenoic acid is an intermediate in a biosynthesis of leukotriene and may be generated from 5-hydroperoxyeicosatetraeonic acid by peroxidase as shown in FIG. 1. 5-oxo-6,8,11,14-eicosatetraenoic acid may be generated by oxidation by 5-hydroxyeicosanoid dehydrogenase (5-HEDH).

[0039] In an exemplary embodiment of the present disclosure, the metabolites via the LO pathway include 5-hydroperoxyeicosatetraenoic acid (5-HpETE), 5-hydroxy-6,8,11,14-eicosatetraenoic acid (5-HETE), or 5-oxo-6,8,11,14-eicosatetraenoic acid (5-oxoETE).

[0040] The AA according to the present disclosure and the metabolic products thereof, that is, the metabolites or the derivatives may be detected by various methods known in the art and appropriate methods may be selected at a level of those skilled in the art. For example, Applied Biochemistry and Biotechnology Jul-Sep 2000, Volume 88, Issue 1-3, pp 33-44; E.J. Want et al. Nature Protocols 2010; 5: 1005 - 1018 and Stanke-Labesque F et al. J Allergy Clin Immunol 2009;124:364-70 may be referred.

[0041] In an exemplary embodiment of the present disclosure, an arachidonic acid and a derivative thereof are detected by using a receptor, a ligand, a substrate, an antibody, an antibody fragment, an aptamer, an avidity multimer, or peptidomimetics which specifically recognizes the arachidonic acid and the derivative thereof of the present disclosure. The detection material may be used in an antibody analysis method, a chemiluminescence analysis method, a liquid chromatography, mass spectrometry method and the like, which are of course, may be used for detecting the biomarker according to the present disclosure.

[0042] According to an exemplary embodiment of the present disclosure, the biomarker may be detected by using mass spectrometry which may for example be found in Applied Biochemistry and Biotechnology Jul-Sep 2000, Volume 88, Issue 1-3, pp 33-44.

[0043] In another exemplary embodiment, methods employing antibodies may be used. The methods are using materials that specifically recognize the AA or the derivative thereof of the present disclosure, which include for example polyclonal antibodies, monoclonal antibodies, receptors, ligands, antibody fragments, antibody mimetics, aptamers, avidity multimers, or peptidomimetics. The immunoassays using sandwich system like ELISA (Enzyme Linked Immuno Sorbent Assay), or RIA (Radio Immuno Assay) and the like may be used for quantitative and/or qualitative detection of the present markers. In this system, the biological samples are reacted with a first antibody fixed to a solid substrate/support such as a glass, a plastic (for example, polystyrene), polysaccharides, a bead, a nylon or nitrocellulose membrane or a microplate well to form a complex and the complex is then allowed to react with an second antibody that is usually labeled with agents that can be detected directly or indirectly such as radioactive substances like ^{3}H or ^{125}I, fluorescent materials, chemiluminescent substances, hapten, biotin, or digoxygenin and the like. In some cases, the labeling materials are conjugated with an enzyme such as horseradish peroxidase, alkaline phosphatase, or maleate dehydrogenase that is able to produce colors or color changes or illuminate in the presence of appropriate substrates.

[0044] Other methods based on immune reaction may also be used. In other embodiment, an Immuno Electrophoresis such as an Ouchterlony plate, a Western blot, a Crossed IE, a Rocket IE, a Fused Rocket IE, or an Affinity IE, which can detect the markers simply by antigen-antibody reaction may be used.

[0045] The agents or materials that may be used in the methods described above are known the art. For example,

the markers may be detected through an antigen-antibody reaction, or a reaction with a substrate, nucleic acid or peptide aptamers, receptors or ligands that specifically recognize the present markers, or cofactors or using mass spectrometry.

[0046] The agents or materials that bind or interact specifically with the markers of the present disclosure can be utilized by means of chip or with nanoparticles. The immunoassay or immunostaining methods as described above are disclosed in the following literatures : Enzyme Immunoassay, E. T. Maggio, ed., CRC Press, Boca Raton, Florida, 1980; Gaastra, W., Enzyme-linked immunosorbent assay(ELISA), in Methods in Molecular Biology, Vol. 1, Walker, J.M. ed., Humana Press, NJ, 1984 etc. The intensities of the signals generated by the immunoassay mentioned above are then analyzed, namely compared with the signals from appropriate controls for the determination whether the sample is related to hypoxia.

[0047] In another aspect, the present disclosure relates to a kit for diagnosing hypoxic diseases including the composition according to the present disclosure. The kit of the present disclosure can be used for diagnosis or prognosis or progression of hypoxia or hopoxia related diseases by quantitative and/or qualitative detection of the present markers, the AA and the metabolite thereof in biological samples of interest.

[0048] The kit of the present disclosure may be used for the aforementioned various detecting methods. For example, the kits of the present disclosure may be formulated as an immunochromatography strip kit, an ELISA kit, a chemiluminescence analysis kit, a luminex kit, and the like. The ELISA kit includes a specific antibody to the biomarker. The antibody employed has a high specificity and affinity to each biomarker and almost no across-reactivity against other biomarkers. Such antibodies are a monoclonal antibody, a polyclonal antibody, or a recombinant antibody. Further, the ELISA kit may include an antibody specific to a control sample. Further, ELISA kits may include reagents capable of detecting the antibody bound to the marker, for example, labelled secondary antibodies, chromophores, enzymes (for example, conjugated to antibodies), and substrates thereof or other materials capable of binding to antibodies.

[0049] The luminex kit, as a high-throughput quantitative analysis method capable of simultaneously processing a maximum of 100 different kinds of analytes while employing a small amount (10 to 20 μl) of sample which is not pretreated, is an analysis method having a high sensitivity (pg unit) and short analysis time, and can replace an existing ELISA or ELISPOT. The luminex assay, as a multiplex fluorescence microplate assay capable of simultaneously processing more than 100 different kinds of biological materials in each well of a 96-well plate, distinguishes and quantifies more than 100 different color groups of polystyrene beads by performing signal transfer in real time by using two kinds of laser detectors. The 100 beads may be constituted to be distinguished in the following ways. At one side, red fluorescence beads are divided into 10 levels or more according to their fluorescent intensity and at the other side, orange fluorescence beads are divided into 10 levels or more, and the beads therebetween shows a unique intensity according to the mixed ratio of the orange and red fluorescence thus generating 100 color-coded bead sets. Further, an antibody specific to the maker to be analyzed is attached to each bead and thus the biomarkers may be quantified by an immune antibody reaction using the beads.

[0050] The luminex kit capable of performing the luminex assay of the present disclosure includes a specific antibody to the biomarker. The antibody employed has a high specificity and affinity to each biomarker and almost no across-reactivity against other biomarkers. Such antibodies are a monoclonal antibody, a polyclonal antibody, or a recombinant antibody. Further, the luminex kit may also include a specific antibody to a material in a control group. Other luminex kits may also include reagents capable of detecting the antibody bound to the marker, for example, labelled secondary antibodies, chromophores, enzymes (for example, conjugated to antibodies), and substrates thereof or other materials capable of binding to antibodies. The antibody may be an antibody conjugated to microparticles and further, the microparticle may be colored latex or a colloidal gold particle.

[0051] In the kit for diagnosing the hypoxic disease or analyzing the prognosis thereof of the present disclosure, a kit for diagnosing hypoxic disease including an immunochromatographic strip for diagnosis may be a rapid test or diagnosis kit including essential elements required for performing a rapid test which may show the result within 5 minutes. The immunochromatographic strip may include (a) a sample pad to which a sample is absorbed; (b) a binding pad which is binding to a biomarker in the sample; (c) a reaction membrane in which a reaction line including monoclonal antibodies for the biomarker and a control line are formed; (d) an absorption pad to which the residual sample is absorbed; and (e) a backing or supporting material. The antibody employed has a high specificity and affinity to each biomarker and almost no across-reactivity against other biomarkers. Such antibodies are a monoclonal antibody, a polyclonal antibody, or a recombinant antibody. Further, the rapid test kit may include an antibody specific to a control material. Other rapid test kits may include other materials required for diagnosis such as reagents capable of detecting the bound antibodies, for example, a nitro cellulose membrane to which the specific antibody and the secondary antibody are fixed, a membrane coupled with the beads bound to the antibody, the absorption pad, and the sample pad.

[0052] In yet another aspect, the present disclosure further provides a method of detecting a hypoxic marker comprising quantitative and/or qualitative detection of the AA and/or the derivative in the biological material from the subject to detect/diagnose hypoxia, diagnose the hypoxic disease and/or monitor the prognosis of hypoxia or to provide information required therefor.

[0053] The biological samples are separated from the subject and include blood, plasma, serum, cerebrospinal fluid,

hair, tissue, cell and urine. In the exemplary embodiment of the present disclosure, the hair separated from the subject or the urine, particularly, a first morning urine may be used.

[0054] Accordingly, the present methods can be advantageously used to diagnose or prognosis hypoxia or a disease accompanied by hypoxia by performing qualitative and quantitative analysis of the AA and the derivative thereof using the methods as described above and comparing the analysis results with an appropriate control group.

[0055] According to one exemplary embodiment of the present disclosure, the urine of the hypoxia patient is obtained and the amount of arachidonic acid, 5-HpETE, 5-HETE, or 5-oxo ETE in the urine is measured in the samples of each patient by performing a liquid chromatography/mass spectrometer (LC/MS) method, and then the measured value may be used for diagnosis and/or prognosis of hypoxia in comparison to a control group.

[0056] In the present disclosure, the control is a sample from a person who does not suffer from the hypoxia-related disease for example, obstructive sleep apnea in which the controls are divided into a simple snoring group (AHI < 5) and a obstructive sleep apnea group (AHI > 5) based on an apnea-hypopnea Index (AHI), and the simple snoring groups are used as a control. Or apnea patients (for example, primary insomniac patients) other than obstructive sleep apnea who have AHI < 5 and no snoring may also serve as a control group.

[0057] As an example, the cut off (upper limit in the case of increasing biomarker/the lower limit in the case of decreasing biomarker) value in a normal range for a particular marker is determined in a control group, which is then used for diagnosis of the subject suspected of the disease and the subject may be diagnosed as hopoxia when the value is increased by about 50% or more compared to cut off value. Particularly early diagnosis of serious hypoxia is possible when the amount of corresponding biomarker is increased by about 2 times or more as compared to cut off value. However, the value is not limited thereto, and may be different depending on the type of a specific material that is used for detecting hypoxia or diagnosing hypoxia-related diseases. For example, when blood or urine is used as a sample, there is high probability the markers are concentrated therein, and thus, the increased value may be large. Thus the values may be determined in consideration of the factors as described above. In addition, by determining whether the amount of corresponding biomarkers in a patient with hypoxia is returned to a normal range after treatment, it is possible to determine and monitoring the efficacy of the therapy used. The diagnosis of hypoxia using the biomarker according to the present disclosure may be used alone or in combination of other known methods.

[0058] Hereinafter, the present disclosure will be described in detail with reference to Examples. However, the scope of the present disclosure is not limited by these Examples.

Example 1: Selection of biomarker

[0059] The biomarkers were selected through analyzing cells (Example 1-1) and samples from the subjects (Example 1-2) as described below.

Example 1-1: Selection of biomarkers using THP-1 cell line

[0060] A THP-1 cell line, a human monocyte-derived cell line was cultured in the conditions of regular oxygen (21% oxygen), low oxygen (1% oxygen), and regular oxygen after low oxygen (reoxygenation), and then, the metabolites included in the culture media were identified and quantified using a LC/Q-TOF MS analysis as follows.

[0061] The present Example is to identify the changes in the metabolites in the bodily fluids (including blood and urine) under acute hypoxia condition by exposing the blood cells to various low oxygen conditions

① Cell culture

[0062] The human-derived monocyte cells (THP-1) (Korean Cell Line Bank, KCLB No. 40202) at 5×10^5 cells/4 mL medium were cultured in DMEM (Dulbecco's modified Eagle's medium) including 10% FBS (fetal bovine serum) under the condition of regular oxygen (21% O_2, 5% CO_2) at 37°C for 24 hours, the condition of low oxygen (1% O_2, 5% CO_2) at 37°C for 24 hours, or the condition of regular oxygen for 16 hours after low oxygen for 8 hours (reoxygenation) at 37°C.

[0063] After that, the media without the cells were isolated to obtain the samples to be analyzed. The media were subjected to a centrifugation at 3000 rpm and 4°C to obtain supernatants only. The supernatants were then aliquoted and stored in a -70°C cryogenic deep-freezer, and were thawed immediately before use.

[0064] In order to decrease the expression of a hypoxia-inducible factor-1 (HIF-1), a major regulator in a low oxygen environment, two types of si-HIF-1alpha (#1, 5'-CAAAGUUAAAGCAUCAGG-3'; #2, 5'-UGUACUGUCCUGUGGUGA-3') were prepared, and then used for transfection into the cells using Lipofectamine RNA iMAX reagents (Life Technologies, USA) following the manufacturer's instruction. In addition, 2-methoxyestradiol (2ME2) and 17-N-Allylamino-17-demethoxygeldanamycin (17-AAG) used (Selleck chemicals) as the inhibitors of HIF-1 were used, and YC-1 was purchased from A.G. Scientific. The cells were treated also with 2ME2 and 17-AAG at the concentrations of 100 μM during the exposure of a low oxygen condition.

② Preparation of assay sample

[0065] H_2O (400 μl) at 4°C and medium (100 μl) were put into a 1.5 ml micro tube so as to be diluted. After that, the sample was mixed well using a vortex,, and then was filtered/centrifuged at 4°C and 14,000 g for 20 minutes to obtain the supernatant thereof. The supernatant was collected in assay vials. In addition, for a QC (quality control) assay, the diluted samples of 50 μl from all the samples were mixed (pooling).

③ Analysis of metabolite by LC/Q-TOF MS assay

[0066] The samples that were prepared as described above were isolated by the system equipped with a reverse column, Zorbax SB-C18, 50 x 2.1 mm, 1.8 μm (Agilent Technologies, USA) in Binary Agilent 1200 series HPLC (Agilent Technologies). At this time, the assay sample in the amount of 5 μl was injected so as to pass through the column that was heated at 40°C. The metabolite sample was subjected to a gradient elution with 98% solvent A (2 mM ammonium formate and 0.1% formic acid in H_2O) and 2% solvent B (0.1% formic acid in methanol) at the rate of 400 □ 1/min for 21 minutes. In order to avoid a cross-contamination, a blank run was performed between the sample runs.

[0067] ESI algorithm was used by coupling an Agilent 6530 quadrupole time-of-flight (Q-TOF) mass spectrometer (Agilent Technologies) with HPLC. Four centroid data per one second for one spectrum from 100 to 1100 m/z were obtained, and the m/z for all the spectra were calibrated in real time using the reference value externally supplied.

[0068] The metabolites were first identified from the mass spectra in the HMDB (http://www.hmdb.ca/) and METLIN (http://metlin.scripps.edu/) database using a software (Agilent MassHunter Qualitative Analysis (version B.05.00), Agilent Mass Profiler Professional (version B.02.02)). After that, the cleavage pattern by the LC/MS/MS assay of the corresponding metabolite was subjected to an additional confirmation work as compared to a practical standard material (Cayman, Ann Arbor, MI, USA).

④ Quantitative analysis of metabolite using LC/Q-TOF MS

[0069] The relative concentration of the corresponding metabolites was calculated using the chromatograph area of the respective metabolite in the culture solution obtained from the corresponding cells.

[0070] The metabolites that were significantly increased in the culture solution subjected to a low oxygen condition as compared to the cell culture solution under a normal oxygen condition were screened, and two metabolites, 5-HETE and 5-oxoETE, were statistically and significantly increased under the condition of low oxygen. For this reason, 5-HETE and 5-oxoETE, and also, AA and 5-HpETE, which were high rank metabolites thereof were selected as a biomarker.

Example 1-2: Selection of biomarker using subjects to be tested

Example 1-2-1: Recruitment of subjects to be tested and setting experimental group/control group

[0071] The patients in ages of 10 to 60 that visited the otolaryngology clinic in Seoul University Hospital and the Seoul Sleep Center with snoring and sleep apnea syndrome and who agreed to the research were selected as the subjects and subjected to polysomnography. The patients who had a history of tumor or were unsuitable for an operation and polysomnography or could not provide urine due to a kidney disease were excluded. Simple snoring patients (AHI < 5) and the patients with sleep apnea syndrome (AHI > 5) were determined based on an apnea-hypopnea index (AHI) after performing polysomnography. Using the simple snoring patients or insomnia patients as a control group, the results of the experimental group, i.e., the patients with sleep apnea syndrome, were compared and analyzed.

[0072] In the present Example, the corresponding metabolite changes were measured in the urine sample of the patient with obstructive sleep apnea syndrome, the average disease period of whom generally extends from several years to several decades to determine the changes in the metabolites in the bodily fluid of the chronic hypoxia.

[Table 1]

Group	N	Age	BMI	AHI	LowSat
Control	6	35.8±5.9	24.1±2.3	1.4±1.7	92.3±1.6
Mild OSA	17	40.5±6.8	24.8±2.6	17.8±9.5	86.1±3.3
Severe OSA	20	39.3±8.1	27.1±2.8	49.6±21.1	73.2±5.3
P-value*	-	0.087	0.003	<0.001	<0.001

*Kruskal Wallis Test

[0073] In both the experimental groups and control groups, the basic information of a patient, such as, sex, age, height, body weight, neck size, and waist size was determined, and the results of a basal blood test (CBC, admission panel, and the like) and a radiographic test (cephalometry) which are a test required for patients with sleep apnea syndrome were also collected.

[0074] An apnea-hypopnea index (AHI) is an index for determining severity of sleep apnea syndrome, and represented as the numbers of apnea and partial breathing (hypopnea) per hour of sleeping. The patients were classified as normal when the number is 0 to 4, as mild when 5 to 14, as moderate when15 to 30 and as severe state when over 30.

Example 1-2-2: Selection of biomarkers

[0075] Various indices were collected by performing polysomnography for determine the development and severity of sleep apnea syndrome of the patients and control groups. In addition, the biomarkers that were increased or decreased were screened in the patient groups by identifying and quantifying the metabolites in the first morning urine from the patient groups and control group using a mass spectrometric assay as described below. Finally, AA, and 5-HpETE, 5-HETE, and 5-oxoETE were selected as a biomarker based on the correlation between the candidate biomarkers and the major indices obtained from, such as, clinical symptoms and polysomnography.

[0076] The correlations among the relative quantitative value which was obtained by calibrating the respective biomarker with the amount of creatine present in the urine, and the lowest oxygen saturation (95% or more as a normal value; the lower value indicates severe sleep apnea syndrome) or AHI (higher value means more severe sleep apnea syndrome) that is an severity index of sleep apnea syndrome determined by polysomnography were analyzed using a Pearson's correlation test as known.

[0077] The metabolites included in the urine of the subject to be tested were identified and quantified as described in Example 1-1 using a LC/Q-TOF MS analysis except that the concentration of urine in the metabolite quantitative analysis using LC/Q-TOF MS was calibrated with creatinine. The relative concentration of the metabolite was calculated by dividing the chromatogram area of respective metabolite with the chromatogram area of creatinine.

[0078] It was confirmed that the arachidonic acid and the derivative thereof were significantly increased in both experiments as described in Examples 1-1 and 1-2 (see FIGs. 1 to 6 and FIGs. 8 to 10).

[0079] In detail, it was confirmed that 5-HETE and 5-oxoETE, two types of arachidonate derivatives, were increased in Example 1-1, and an arachidonic acid, 5-HpETE, 5-HETE, and 5-oxoETE were increased in Example 1-2. In the case of Example 1-2, it was thought that since the disease period was generally several years, or 10 years or more, the stimulation by low oxygen was acting for a longer period of time ("chronic"), and thereby, in addition to 5-HETE and 5-oxoETE, an arachidonic acid or a precursor, such as, 5-HpETE were also increased.

[0080] The results of Example 1-1 testing the effect of only the low oxygen stimulation to find the biomarkers under the low oxygen condition and Example 1-2 testing the changes of the metabolites in the patient experiencing the low oxygen condition, all support that the present markers can be used advantageously to determine or diagnose hypoxia.

Example 2: Verification of the biomarkers

Example 2-1: Verification of the biomarker selected using cell lines

[0081] After THP-1 cells were cultured under the conditions of normal oxygen level, low oxygen level, and normal

oxygen level after the low oxygen level (reoxygenation) as Example 1-1, AA, and 5-HpETE, 5-HETE, and 5-oxoETE in the metabolites included in the culture solutions was quantified using a LC/Q-TOF MS analysis as Example 1.

[0082] The results are shown in FIG. 2, and all of the markers were metabolites induced or derived from 5-HpETE through an enzymatic function of 5-lipoxygenase (5-LO) and peroxidase in AA and are derivatives of an arachidonic acid (AA).

[0083] Then, using the method described in Example 1-1, the expression of a hypoxia-inducible factor-1 (HIF-1) that was a major regulator under the low oxygen environment was inhibited using si-RNAs (si-HIF-1a#1 and si-HIF-1a#2), and then, the metabolites were analyzed. The results are shown in FIG. 3. As in FIG. 3, it was confirmed that 5-HETE and 5-oxoETE that were increased under the low oxygen condition (H) were decreased. Similarly, it was confirmed that even when the cells were treated with the inhibitors of HIF-1, 2-methoxyestradiol (2ME2), 17-N-Allylamino-17-demethoxygeldanamycin (17-AAG), and YC-1, 5-HETE and 5-oxoETE that were increased under the low oxygen condition (H) were decreased. It means that 5-HETE and 5-oxoETE that were increased under the low oxygen condition (H) were increased HIF-1-dependently. Especially, it was confirmed that considering that HIF-1 is a major regulator during low oxygen adaptation and metabolic regulation, the increases of 5-HETE and 5-oxoETE under the low oxygen condition reflect the changes that occurs specifically in the body under low oxygen condition. The HIF-dependent increase indicates that the increases of the metabolites according to the present disclosure are closely related to the low oxygen environment.

[0084] In addition, the results that were confirmed in THP-1 cells, a human-derived blood cell line, were reconfirmed using a primary human polymorphonuclear cell from PromoCell, Germany. As a cell medium, Mononuclear Cell Medium (C-28030) manufactured by PromoCell was used, and the analysis was performed as described in Example 1. The results are shown in FIG. 5. As in FIG. 5, it was confirmed that as compared to the normal oxygen condition, 5-HETE and 5-oxoETE under the condition of low oxygen for 8 hours and intermittent low oxygen for 8 hours (IH8, the culture was subjected to 1% oxygen condition for 30 minutes, and then, 21% oxygen condition for 30 minutes per hour) were increased.

[0085] In addition, it was confirmed that in the case of culturing under the normal oxygen condition for 16 hours after the low oxygen condition for 8 hours (H8R16), 5-HETE and 5-oxoETE were also increased. 5-oxoETE was also increased even in the case of culturing under the normal oxygen condition for 16 hours after the intermittent oxygen condition for 8 hours (IH8R16).

[0086] In addition, as shown in FIG. 6, it was confirmed that when HIF-1 alpha in the human primary polymorphonuclear as in THF-1cells was inhibited by the introduction of si-RNA, 5-HETE and 5-oxoETE that were increased under the low oxygen condition were decreased. These results indicate that the increases of 5-HETE and 5-oxoETE under the low oxygen condition depend on HIF-1.

[0087] In the 5-LO metabolic pathway of an arachidonic acid (refer to FIG. 1), considering that the increase starts from 5-HETE under the low oxygen level, it is determined that the process of converting 5-HpETE to 5-HETE was activated under the low oxygen condition. For this reason, the activity of Glutathione Peroxidase (GPX) that is an enzyme for converting 5-HpETE into 5-HETE was measured. The GPX activity was measured using a GPX assay kit (ab102530, Abcam, USA), and the GPX activity measurement principle of the kit was as follows. First, the GPX allows reduced glutathione (GSH) to be converted into oxidized glutathione (GSSG). When the GSSG is again reduced into GSH by glutathione reductase (GR), NADPH was consumed. Then the absorbance at 340 nm is measured using a spectrophotometer so as to measure the level of the decrease of NADPH, which then is used to calculate the activity of GPX.

[0088] The results are shown in FIG. 7. As in FIG. 7, it was confirmed that when the cells were cultured under the low oxygen condition for 24 hours (H24) and the normal oxygen condition for 16 hours after the low oxygen condition for 8 hours (H8R16), all the activities of GPX were increased.

[0089] These results indicate that the hypoxia can be detected or diagnosed by determine the concentrations of an arachidonic acid, 5-HpETE, 5-HETE, and 5-oxoETE markers according to the present disclosure.

Example 2-2: Verification of biomarkers in patient and control group

[0090] The metabolites in the patients with obstructive sleep apnea syndrome and the control group were tested using the methods as described in Example 1-2-2, and the correlation of the lowest oxygen saturation collected from polysomnography that was measured at the same day was determined using a Pearson's correlation test. The metabolites were calibrated with creatinine concentration in the urine, and quantified.

[0091] As a result in FIGs. 8 to 10, it was confirmed that the lower the oxygen saturations levels are, the higher the level of all the markers, i.e., an arachidonic acid, 5-HpETE, 5-HETE, and 5-oxoETE metabolites are. Especially, 5-HETE and 5-oxoETE showed a close correlation with the lowest oxygen saturation. This indicates that 5-HETE and 5-oxoETE are increased more in severe sleep apnea syndrome patient who are exposed under a low oxygen condition for a longer period of time than the less severe patient.

[0092] In other words, it was confirmed that as the metabolisms are progressed, arachidonic acid, 5-HpETE, 5-HETE, and, 5-oxoETE in the urine of the patient with obstructive sleep apnea syndrome were detected in a higher amount.

Especially, it was confirmed that 5-HETE and 5-oxoETE in the urine of the patient with severe sleep apnea syndrome were detected in a higher amount as compared to the patient with mild sleep apnea syndrome, and thus can be used to determine the severity of a disease.

[0093] Data were represented as an average and standard deviation were used, the difference between different tissues from the same patient was verified using a Wilcoxon signed rank test, a non-parametric testing method, and the differences between the experimental group and control group were analyzed using a Mann-Whitney test. The correlations between the selected biomarkers and the conventional major disease indices were analyzed, and if necessary, a layering analysis was performed.

[0094] While this disclosure has been described in connection with what is presently considered to be practical exemplary embodiments, it is to be understood that the disclosure is not limited to the disclosed embodiments, but, on the contrary, is intended to cover various modifications and equivalent arrangements included within the spirit and scope of the appended claims.

[0095] All the technical terms that were used in the present disclosure, have the same meanings that are understood by a skilled person in this art, unless otherwise specified. The contents of all the publications disclosed in the present description as a reference document are incorporated in the present disclosure.

Claims

1. A method for detecting hypoxia or diagnosing hypoxia-related diseases in a subject, the method comprising:

   providing a biological sample from a subject to be tested;
   detecting an arachidonic acid or a derivative thereof from the biological sample: and
   diagnosing the subject as hypoxia when the amount of the arachidonic acid or the derivative thereof in the biological material is increased as compared to a control group.

2. The method of claim 1, wherein the derivative of the arachidonic acid is a derivative produced in a metabolic pathway of 5-lipoxygenase of the arachidonic acid.

3. The method of claim 1, wherein the derivative of the arachidonic acid is a 5-hydroperoxy eicosatetraenoic acid (5-HpETE), a 5-hydroxy-6,8,11,14-eicosatetraenoic acid (5-HETE), or a 5-oxo-6,8,11,14-eicosatetraenoic acid (5-oxoETE).

4. The method of claim 1, wherein the biological sample is a bodily fluid including whole blood, plasma, serum, saliva, tear, sweat, amniotic fluid, and urine, hairs, cells, and tissues.

5. The method of claim 1, wherein the detection is performed by an antibody analysis, a chemiluminescent assay, or a liquid chromatography/mass spectrometry assay.

6. The method of claim 1, wherein the hypoxia is an acute systemic hypoxia, a chronic systemic hypoxia, an acute local hypoxia, or a chronic local hypoxia.

7. The method of claim 6, wherein the systemic hypoxias is a conditon accompanied by an obstructive sleep apnea, an asthma, a chronic obstructive pulmonary disease (COPD, Lung fibrosis), a pulmonary hypertension and pulmonary edema, a pulmonary thromboembolism, a cardiac failure, an airway obstruction, a pneumothorax, a perinatal asphyxia, an anemia, a hemoglobinopathy, a carbon monoxide poisoning, or cyanide poisoning; and
   the local hypoxias are a condition accompanied by a cerebrovascular diseases a cardiovascular disease, a tumor, or an ischemic tissue damage including hypoxic ischemic encephalopathy.

8. The method of claim 6, wherein the acute hypoxias comprises an asthma, a pulmonary edema, a pulmonary thromboembolism, an airway obstruction, a perinatal asphyxia, a carbon monoxide poisoning, a cerebrovascular or a cardiovascular obstruction or bleeding; and
   the chronic hypoxias comprise an obstructive sleep apnea syndrome, a chronic obstructive pulmonary disease (COPD, Lung fibrosis), a pulmonary hypertension, a cardiac failure, an anemia, a hemoglobinopathy, or a tumor.

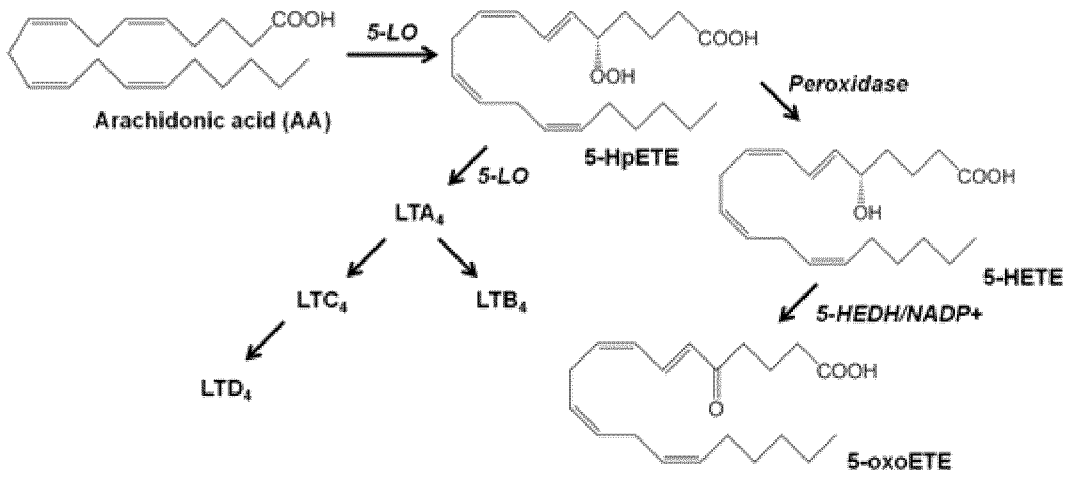
9. The method of claim 1, wherein the hypoxia comprises an acute or a chronic hypoxia; the 5-HETE and/or 5-oxoETE are detected for the acute hypoxias, and the arachidonic acid, 5-HpETE, 5-HETE, and/or 5-oxoETE are detected for the chronic hypoxia.

10. An arachidonic acid or a derivative thereof used for detecting hypoxia or diagnosing hypoxia-related diseases.

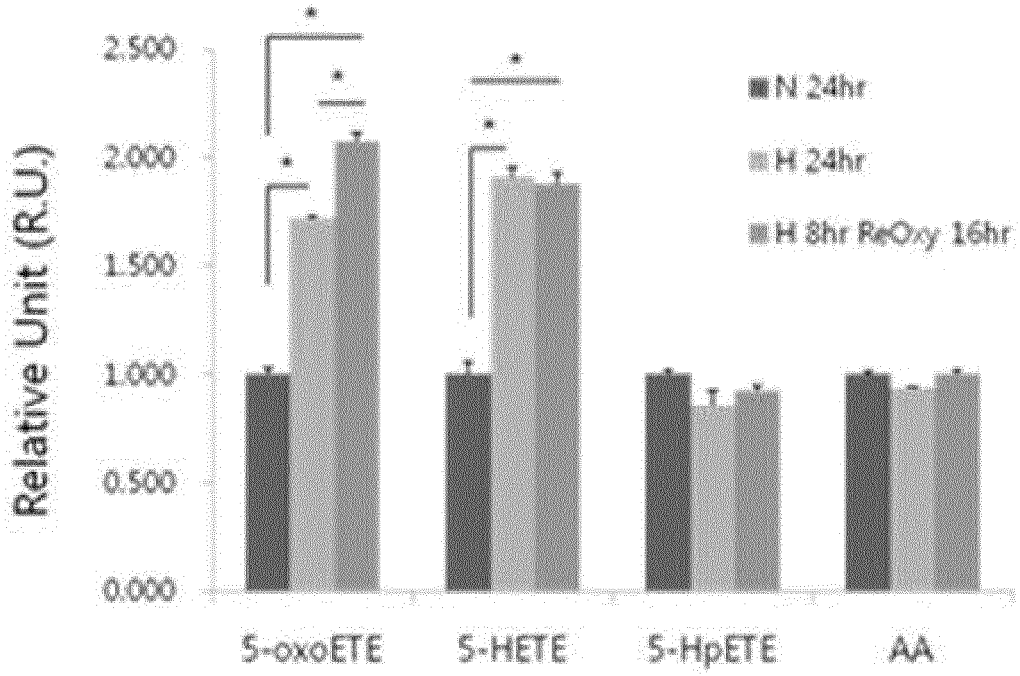
11. The arachidonic acid or a derivative thereof of claim 10, wherein the derivative of the arachidonic acid is a derivative produced in a metabolic pathway of 5-lipoxygenase of the arachidonic acid.

12. The arachidonic acid or a derivative thereof of claim 11, wherein the derivative of the arachidonic acid is a 5-hydroperoxy eicosatetraenoic acid (5-HpETE), a 5-hydroxy-6,8,11,14-eicosatetraenoic acid (5-HETE), or a 5-oxo-6,8,11,14-eicosatetraenoic acid (5-oxoETE).

[FIG. 1]


COOH
5-LO
COOH
OOH
Peroxidase
Arachidonic acid (AA)
5-HpETE
5-LO
COOH
OH
LTA4
5-HETE
LTC4
LTB4
5-HEDH/NADP+
COOH
O
LTD4
5-oxoETE

[FIG. 2]


Relative Unit (R.U.)
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2.000
1.500
1.000
0.500
0.000
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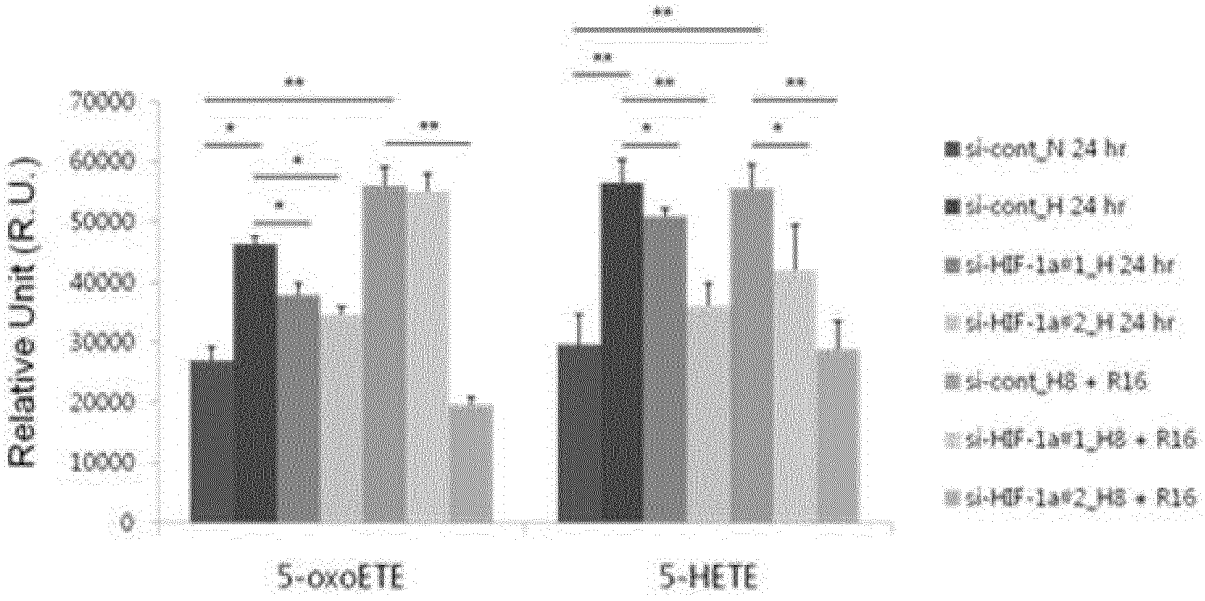
H 24hr
H 8hr ReOxy 16hr
5-oxoETE
5-HETE
5-HpETE
AA

[FIG. 3]


Relative Unit (R.U.)
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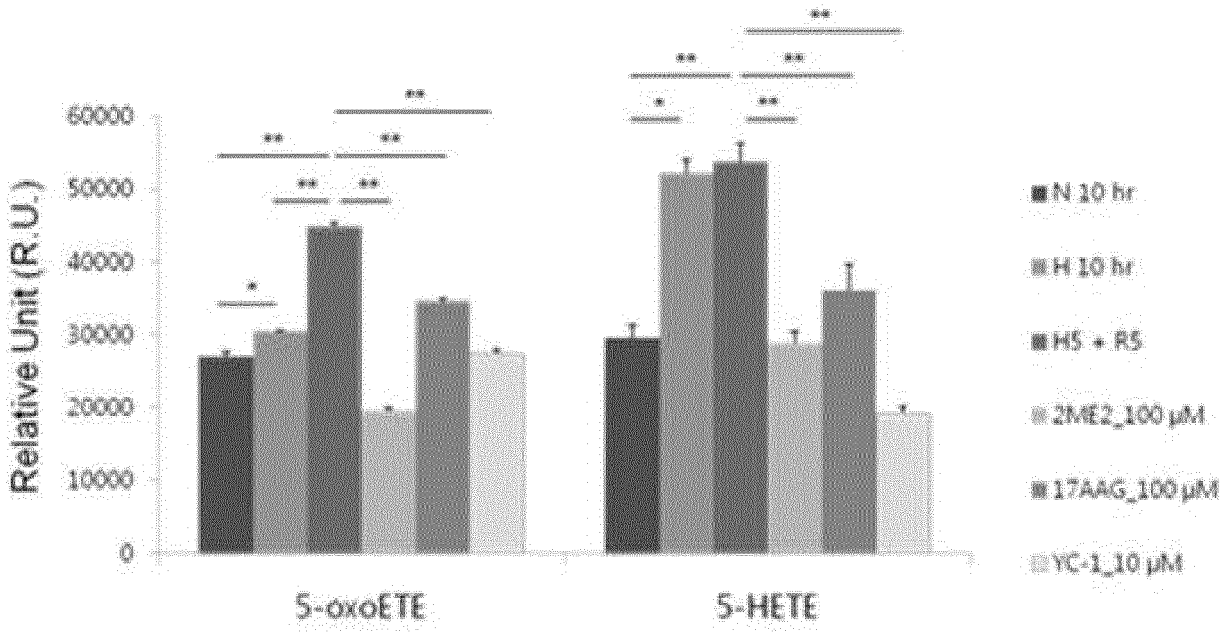
60000
50000
40000
30000
20000
10000
0
5-oxoETE
5-HETE
si-cont_N 24 hr
si-cont_H 24 hr
si-HIF-1a#1_H 24 hr
si-HIF-1a#2_H 24 hr
si-cont_H8 + R16
si-HIF-1a#1_H8 + R16
si-HIF-1a#2_H8 + R16

[FIG. 4]


Relative Unit (R.U.)
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50000
40000
30000
20000
10000
0
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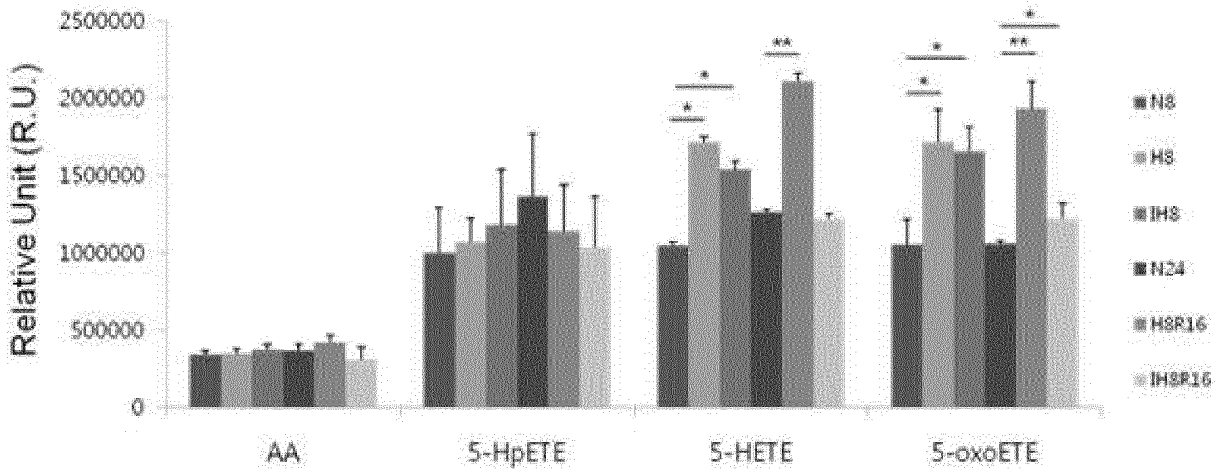
5-HETE
N 10 hr
H 10 hr
H5 + R5
2ME2_100 μM
17AAG_100 μM
YC-1_10 μM

[FIG. 5]


Relative Unit (R.U.)
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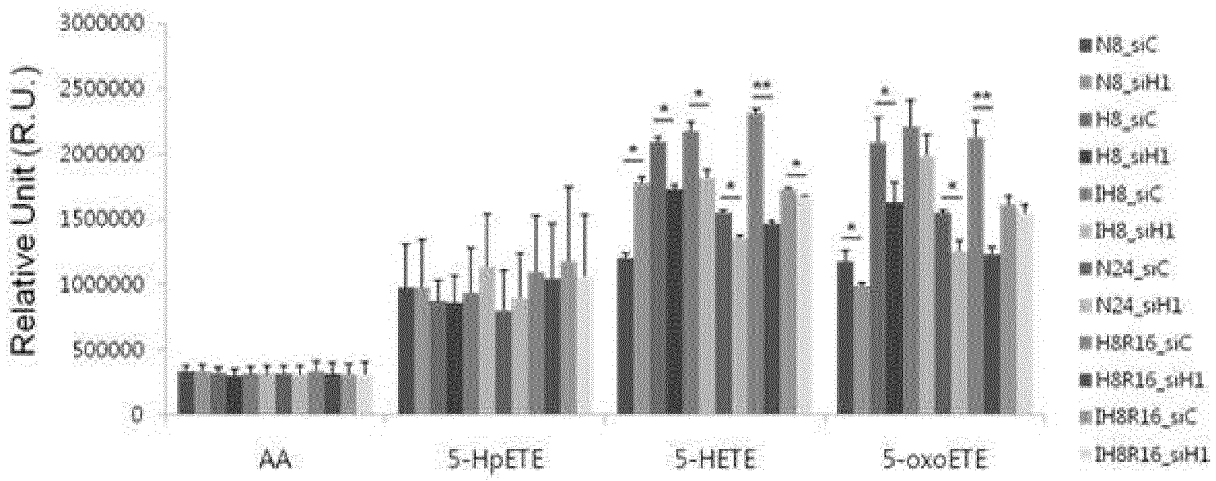
2000000
1500000
1000000
500000
0
AA
5-HpETE
5-HETE
5-oxoETE
N8
H8
IH8
N24
H8R16
IH8R16

[FIG. 6]


Relative Unit (R.U.)
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2000000
1500000
1000000
500000
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AA
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5-oxoETE
N8_siC
N8_siH1
H8_siC
H8_siH1
IH8_siC
IH8_siH1
N24_siC
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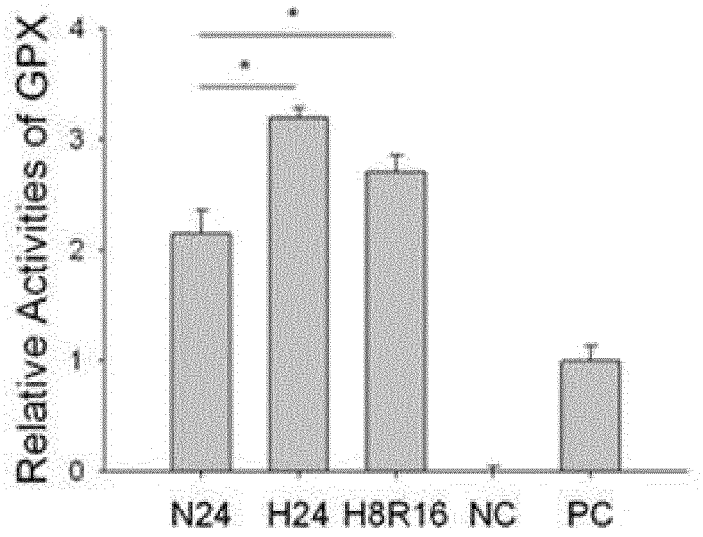
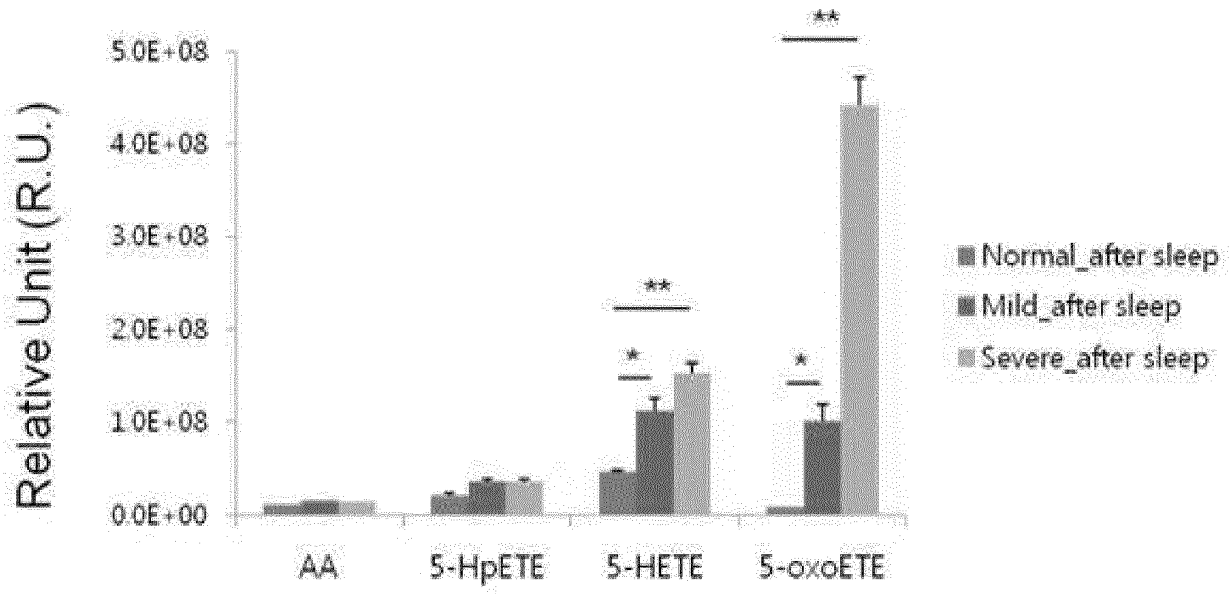
H8R16_siC
H8R16_siH1
IH8R16_siC
IH8R16_siH1

[FIG. 7]



[FIG. 8]


Relative Unit (R.U.)
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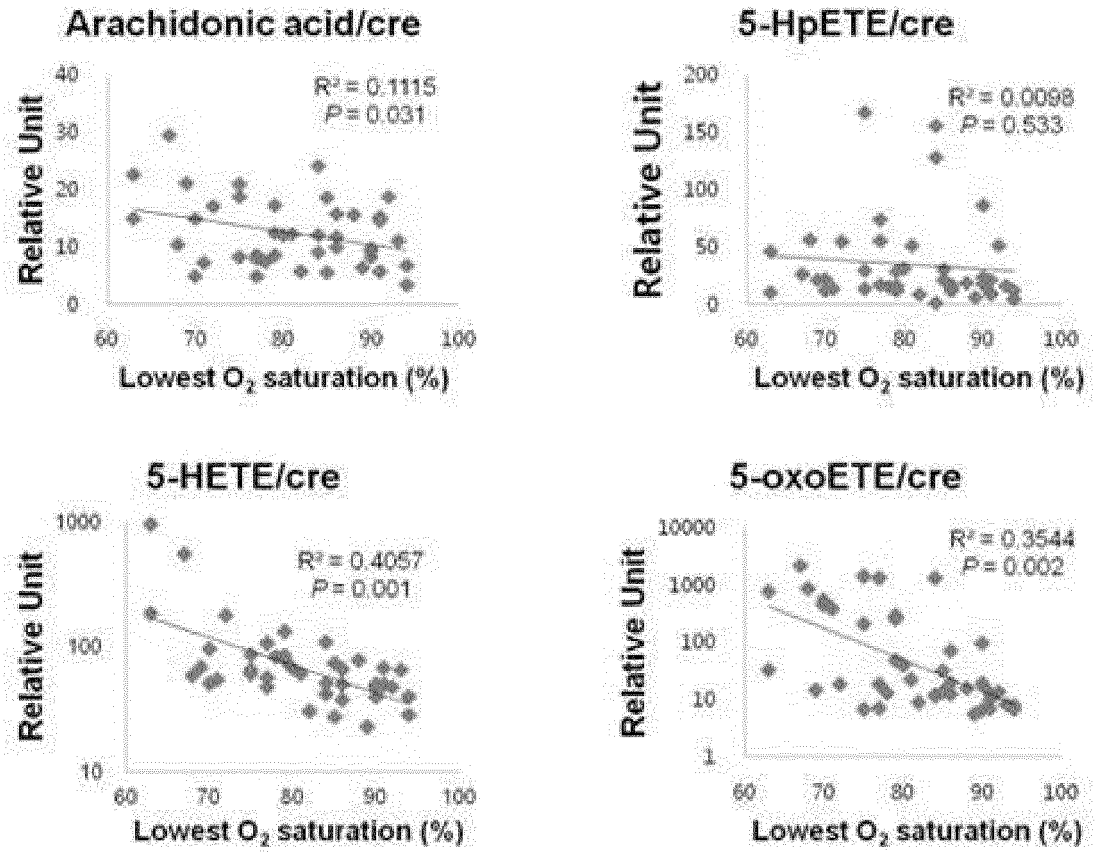
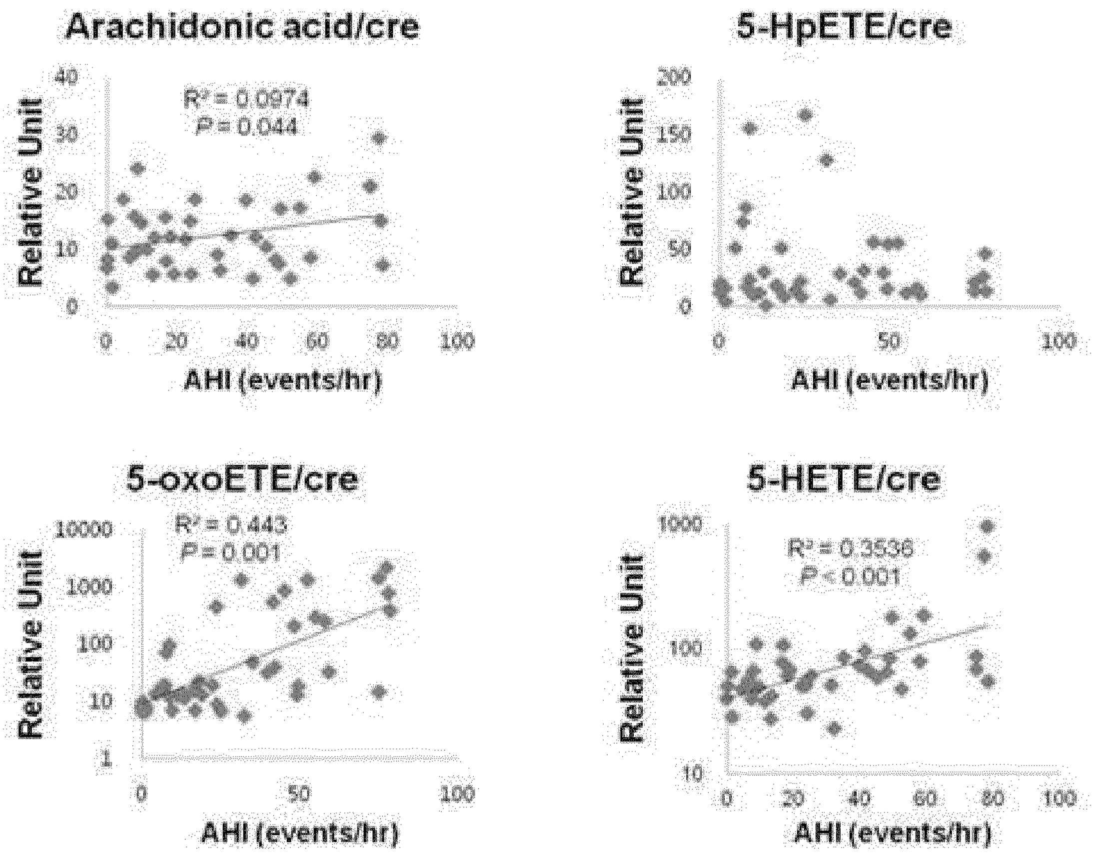
4.0E+08
3.0E+08
2.0E+08
1.0E+08
0.0E+00
AA
5-HpETE
5-HETE
5-oxoETE
**
*
**
*
Normal_after sleep
Mild_after sleep
Severe_after sleep

[FIG. 9]


Arachidonic acid/cre
Relative Unit
R² = 0.1115
P = 0.031
Lowest O2 saturation (%)
5-HpETE/cre
R² = 0.0098
P = 0.533
5-HETE/cre
R² = 0.4057
P = 0.001
5-oxoETE/cre
R² = 0.3544
P = 0.002

[FIG. 10]


Arachidonic acid/cre
R^2 = 0.0974
P = 0.044
Relative Unit
AHI (events/hr)
5-HpETE/cre
Relative Unit
AHI (events/hr)
5-oxoETE/cre
R^2 = 0.443
P = 0.001
Relative Unit
AHI (events/hr)
5-HETE/cre
R^2 = 0.3536
P < 0.001
Relative Unit
AHI (events/hr)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2014/003770

A. CLASSIFICATION OF SUBJECT MATTER

G01N 33/53(2006.01)i, G01N 21/76(2006.01)i, G01N 30/72(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

G01N 33/53; A61K 31/195; G06F 19/00; A61K 31/00; A61K 31/19; A61P 11/00; G01N 21/76; G01N 30/72

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean Utility models and applications for Utility models: IPC as above
Japanese Utility models and applications for Utility models: IPC as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

eKOMPASS (KIPO internal) & Keywords: arachidonic acid, AA, sleep apnea, diagnostic marker, diagnostic kit, 5-lipoxygenase pathway

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2012-0136581 A1 (KELLER, Matthias et al.) 31 May 2012	10
A	WO 2009-063226 A2 (KAROLINSKA INSTITUTET INNOVATIONS AB et al.) 22 May 2009	10
A	WO 2008-054208 A2 (N.V. NUTRICIA et al.) 08 May 2008	10
A	WO 03-017996 A1 (CELLULAR SCIENCES, INC. et al.) 06 March 2003	10

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 AUGUST 2014 (25.08.2014)	**25 AUGUST 2014 (25.08.2014)**
Name and mailing address of the ISA/KR Korean Intellectual Property Office Government Complex-Daejeon, 189 Seonsa-ro, Daejeon 302-701, Republic of Korea Facsimile No. 82-42-472-7140	Authorized officer Telephone No.

Form PCT/ISA/210 (second sheet) (July 2009)

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International application No.
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专利名称(译)	检测缺氧或诊断缺氧相关疾病的方法		
公开(公告)号	EP3009839A4	公开(公告)日	2017-03-01
申请号	EP2014810344	申请日	2014-04-29
[标]申请(专利权)人(译)	首尔大学校产学协力团		
申请(专利权)人(译)	首尔国立大学R＆DB基金会		
当前申请(专利权)人(译)	首尔国立大学研究基金会		
[标]发明人	PARK JONG WAN SHIN HYUN WOO CHO JOO YOUN RHEE CHAE SEO HONG IL HEE		
发明人	PARK, JONG WAN SHIN, HYUN WOO CHO, JOO-YOUN RHEE, CHAE-SEO HONG, IL-HEE		
IPC分类号	G01N33/53 G01N21/76 G01N30/72		
CPC分类号	G01N33/92 C07C57/03 C07C59/42 C07C59/76 C07C409/24 G01N33/6893 G01N2030/8813 G01N2800/52 G01N2800/54 G01N2800/7038		
优先权	1020130068324 2013-06-14 KR 1020130082024 2013-07-12 KR		
其他公开文献	EP3009839B1 EP3009839A1		
外部链接	Espacenet		

摘要(译)

本发明提供用于检测缺氧或诊断缺氧相关疾病的组合物，试剂盒和方法，所述组合物含有用于检测花生四烯酸的材料及其衍生物。根据本发明的组合物，试剂盒和方法可以方便和快速通过检测生物样品中的生物标志物来检测缺氧，因此可用于预防或早期诊断由缺氧引起的疾病，确定疾病的严重程度和治疗效果，跟踪疾病等。