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REVIEW

Biofluids in hypobaric hypoxia: best possible use, investigative strategies and putative markers

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Abstract

Hypobaric hypoxia (HH) is the hypoxia caused by decreased ambient atmospheric pressure (partial pressure of oxygen) as one ascends beyond 2500 m. The entire spectrum of molecular and biochemical studies regarding the effects of HH on an organism heavily depends on biological fluids during exposure to HH. The biological fluids are important sample types. This is because they are easiest to collect using minimally invasive collection procedures, easy to handle and store and low quantities are required for tests/assays. Although many reviews on hypobaric hypoxia in the recent past have been dedicated to the various advances made possible due to the study of biological fluids, biological fluids themselves have never been categorized according to studies done using them. This review concisely outlines the myriad results (particularly translational) in context of hypobaric hypoxia that have been observed in biological fluids, their collection strategies, storage strategies and ethical guidelines. Biological fluidscategorized here are blood (plasma and serum), saliva, tears and urine in context of research on HH and diseases like cancer where hypoxia is an essential condition. This review shall be of help to young investigators for choice of biological samples to be used in their experiments concerning hypoxia related studies and to clinicians involved in translational research providing them a ready reckoner for improving or modifying their approaches.

Keywords Hypobaric hypoxia · High altitude · Human plasma · Saliva · Tears · Urine · Biofluids · Protein markers

Introduction

Hypobaric hypoxia (HH) has been clearly defined in its present form since late 1800 s to mid-1900 s with the radiological and physiological summarization of high-altitude illnesses like high altitude pulmonary edema during the mid-1900s (Hultgren 1969; Hultgren 1970; Hultgren and Marticorena 1978; Antezana et al. 1982). The constant struggle during this period was for assessing symptoms of high-altitude illnesses and understanding their pathophysiology, not to mention the lack of understanding of normal physiological effects of HH exposure, sparked a search for some tangible evidence or object that could be used to decipher various physiological events occurring during HH exposure. This search invariably led to the easily collectible biological fluids (BFs) such as blood and later, saliva, tears and urine. By late 1900 s, one of the early evidences of acclimatization to altitude was increased hematocrit. Although blood plasma and hypoxia were interlinked since mid-1900 s (Berne 1963), as we move further towards the present century, using BFs (particularly plasma), many feats were achieved. Based on initial reports of certain plasma proteins being anti-oxidative in nature (Wayner et al. 1985), the most significant among these has been an exploratory incursion into plasma proteome and genome during HH exposure. Cancer and other diseases which involve hypoxia have also been studied using the plasma proteome of patients (Hanash et al. 2008; Kakisaka et al. 2007; Honda et al. 2005). All of these different studies have led to a huge number of potential protein markers that can be used in clinical diagnosis apart from elucidating the molecular basis of the physiological hypoxic response. Currently the focus is shifting towards other BFs, like saliva, tears and urine, for protein markers of high-altitude illnesses based on observed significant modulations of salivary proteome and urinary peptidome during/after HH exposure (Mainini et al. 2012; Jain et al. 2018). This review

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shall provide concise systematic information regarding the molecular biology and omics (particularly proteomics) based studies on BFs. BFs included in the purview of this article are blood (plasma and serum), saliva, tears and urine. The context of all studies discussed and reviewed in this article will be high altitude and hypoxia. Some aspects of cancer and other diseases that are a result of the hypoxic conditions will also be discussed. This review will also discuss aspects like ethical practices, collection, storage, handling, and best use strategies for BFs in HH based studies.

The ethics of biological fluids (BFs) collection

Bioethics, a domain of study in itself, has remained a focus for biomedical and clinical research since the end of second world war. The basis of the Nuremberg Code (1947) was preventing the reported brutality by German medical teams. Thereafter, in 1966, the International Covenant on Civil and Political Rights stated "No one shall be subjected to torture or to cruel, inhuman or degrading treatment or punishment. In particular, no one shall be subjected without his consent to medical or scientific treatment." This was the first major instance where the consent of an individual to any medical procedures being performed upon them were declared paramount as a right in an international forum. The most recent charter for consent and other ethical considerations is the Helsinki Declaration, duly revised from time to time. Thereafter, in 2005, another landmark document by UNESCO titled "Universal Declaration on Bioethics and Human Rights" was released. Other concerned guidelines like REMARK, CONSORT, STROBE etc. also remain a predominant force in ensuring that only ethical research practices are followed and published across the globe. The basic theme of all these documents, declarations and guidelines is to prevent non-consensual, coercive and unethical exploitation of any research subjects and uphold the dignity and morality of biomedical and clinical research. The above mentioned declarations and guidelines all stress upon complete informed consent of the individual prior to any medical procedure. The collection of blood, saliva, tears and urine amounts to a medical procedure and thus falls under their purview. Thus, apart from general comfort to the subject, their informed consent is mandatory. Any risks pertaining to the procedure are also disclosed explicitly to the subjects. Prior to consent, there must be approval for the study design and methodology by the Institutional Review Board.

Collection of biological fluids

Various collection strategies have been available based on the ingenuity, gathered knowledge and determination of the researchers. However, this review article will focus on the pre-dominant practices observed in the authors lab.

Blood plasma/serum

After clearance of the Institutional Review Committee and consent of the subject, blood is collected from the antecubital area via median cubital or cephalic veins using sterile syringes and capped collection tubes (Fig. 1). The area of extraction is sterilized by alcohol swabs before and after extraction. The collection tubes are either pre-coated with anticoagulants like EDTA/heparin or uncoated. A total of 5 ml (maximum) is collected in a single tube. The blood to be used for extraction of genomic components like RNA is immediately processed using appropriate extraction buffers while the blood for proteomic analyses and other biochemical assays requires separation of either plasma or serum from it. The separation of plasma or serum is carried out immediately. Plasma is separated from whole blood by centrifugation at 3500 rpm for 15 min. Temperature during centrifugation varies from 25 °C (room temperature) to 4 °C (temperature during centrifugation). Serum, on the other hand, requires clotting of the blood in uncoated tubes. After the clot forms in about 20–25 min, the serum is extracted by centrifugation at 3000 rpm for 15 min in refrigerated centrifuge. Immediately following extraction, the supernatant (either plasma or serum) is transferred to fresh polypropylene tubes and protease inhibitor (PI) cocktail is added to them. Throughout these steps, the temperature should be

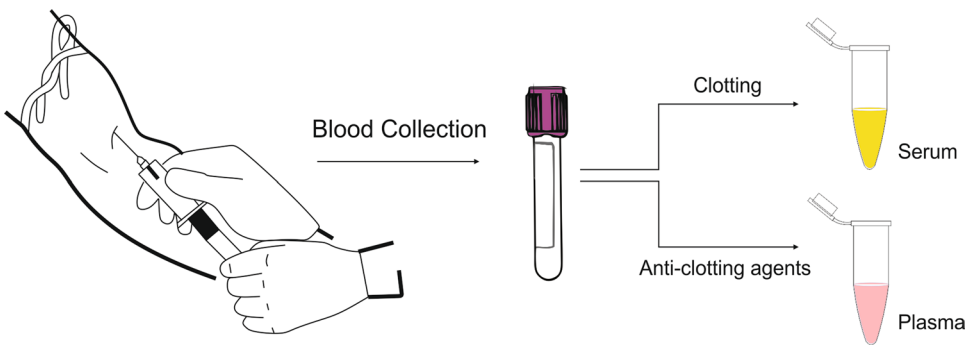

Fig. 1 Collection of blood plasma/serum

kept as close to 4 °C as possible by use of ice buckets. Next step is their proper labeling and storage, as described in next section.

Saliva

Saliva can be collected using various methods such as: A) passive drooling technique, B) suction method, and C) oral swab method (Henson and Wong 2010; Michishige et al. 2006) (Fig. 2). Before saliva collection, inform the subject about the time of saliva collection (optimum time 8–10 a.m.) and ask the subject to refrain from eating, drinking, or oral hygiene procedures for at least 1 h prior to the collection. Give the subject drinking water to rinse their mouth well.

In method A, passive drooling technique or direct expectoration method, subjects are asked to collect saliva in their mouths and to pour it into a pre-chilled sterile tube. The saliva is then centrifuged at 1585×*g* for 15 min at 4 °C to remove insoluble materials, cell debris and other possible contaminants (Jessie et al. 2008). The supernatant is collected and protease inhibitor is added. In method B, suction method, saliva is aspirated using a saliva aspiration set, consisted of an aspiration catheter, a trap and a low-pressure continuous aspirator and collected via the catheter, by gentle continuous suction for 5 min. The saliva is then centrifuged at 1585×g for 15 min at 4 °C to remove insoluble materials, cell debris and other possible contaminants (Jessie et al. 2008). The supernatant is collected and protease inhibitor is added. In method C, oral swab method, using the Salivette collection kits (Sarstedt, Numbrechet, Germany), a neutral, non-covered cotton roll was placed under the tongue of each subject for exactly 5 min and then the roll is returned to a Salivette, followed by centrifugation at 5000g for 5 min at 4 °C. The liquid in the bottom of the tube is collected and protease inhibitor is added.

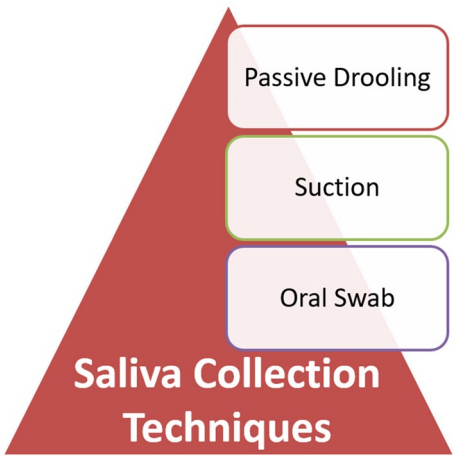

Fig. 2 Techniques for collection of human saliva

Urine

Collection of urine is performed by the steps recommended by The Human Kidney and Urine proteome Project (HKUPP) associated with Human Proteome Organization (HUPO).

Urine specimens can be collected using various techniques: (a) random specimen—it is most commonly used method because it is easiest to obtain and readily available. Also, it can be collected at any time. (b) First Morning Specimen- it is generally more concentrated (due to the length of time the urine is allowed to remain in the bladder) containing relatively higher levels of proteins. The first morning specimen is collected when the patient first wakes up in the morning, having emptied the bladder before going to sleep (~8 h). (c) Midstream clean catch specimen—it is the preferred type of specimen because of the reduced incidence of cellular and microbial contamination. In this method, patients are required to first cleanse the urethral area and then void the first portion of the urine stream into the toilet followed by midstream collection into a clean container (any excess urine should be voided into the toilet). This method of collection can be conducted at any time of day or night. (d) Timed Collection Specimen- it is collected to measure the concentration of substances in urine over a specified length of time, usually 8 or 24 h. In this collection method, the bladder is emptied prior to beginning the timed collection. Then, for the duration of the designated time period, all urine is collected and pooled into a collection container, with the final collection taking place at the very end of that period. (e) Catheter Collection Specimen- it is an assisted procedure conducted when a patient is bedridden or cannot urinate independently. The healthcare provider inserts a foley catheter into the bladder through the urethra to collect the urine specimen into an evacuated tube or transferred from a syringe into a tube or cup. (f) Suprapubic Aspiration Specimen- it is used when a bedridden patient cannot be catheterized or a sterile specimen is required. The urine specimen is collected by needle aspiration through the abdominal wall into the bladder (Fig. 3).

Once collected, urine specimen is processed by centrifugation at 10,000×*g* for 10 min to remove cells and debris (Thomas et al. 2010). The supernatant is transferred in a fresh tube. There is no requirement of addition of protease inhibitor in the collection of routine urine sample. Protease inhibitor is not required as it may change pH and degrade proteases normally present in urine (Zhou et al. 2006; Havanapan and Thongboonkerd 2009).

Tears

Collection of tear fluid is performed by placing a Schirmer's tear test strip on the lower lid of the eye. The strip is allowed

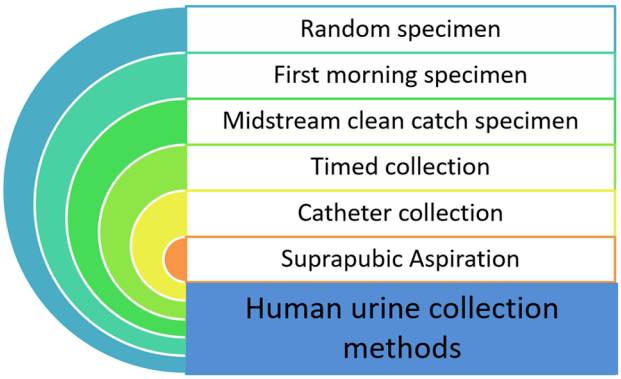

Fig. 3 Techniques for collection of human urine

to sit for 5 min (Stevens 2011). Then, the strip is transferred to 500 µl of 0.1 µm filtered phosphate-buffered saline (PBS) (Aqrawi et al. 2017) (Fig. 4).

Storage of biological fluids

All hypoxic exposure samples require extremely quick handling and storage as the redox status changes upon prolonged exposure to oxygenated environment. Hence, in case of all hypoxia exposed biological fluids, after collection every minute is crucial and the faster the cryogenic conditions are imposed on these samples, the better their performance during lab assays. Non-hypoxic samples do not require immediate cryogenic preservation of their redox status.

Plasma/serum/saliva

Storage of plasma, serum and saliva is almost identical. Immediately after adding PI cocktail and aliquoting the samples, they should be labeled precisely. The label should mention sample details on the tube, e.g. Sample ID, aliquot number, name of investigator, date and the box of samples should have contact number and name of investigator, apart from sample IDs in the box, to inform them of any logistical issues such as malfunctioning of refrigerators. The same should be noted in a project register/workbook of samples.

After thorough completion of labeling, the samples can be stored at either − 20 °C or − 80 °C depending on their period of use. Some samples require to be used within a few weeks or less and these will require multiple freeze/thaw cycles. Such samples are kept at − 20 °C for frequent re-use and to avoid more extreme freeze/thaw cycles. On the other hand, some samples require longer duration of storage and lesser frequency of use or may be required in techniques involving very fragile phenomena (e.g. study of post translational modifications in proteome). These samples must be stored at − 80 °C so that their features and characteristics to be assayed are retained as much as possible. In other cases, where regular biochemical assays like TBARS are to be performed on the samples within 24 h, storage at 4 °C should suffice but is not ideal. However, in all cases, snap freezing (using liquid nitrogen) after aliquoting the samples is recommended.

Urine

The supernatant/sample should be frozen within 4 h of collection or addition of preservatives such as sodium azide or boric acid to the sample should be done prior to freezing. The labeling is done according to the recommendations given by HKUPP and HUPO. They have suggested the 19 sample identifiers describing the patient, processing conditions, storage and data generated from other methods of urinalysis. The samples are then stored at – 80 °C for long term storage (Thomas et al. 2010). Urinary proteins can also be adsorbed on a piece of membrane for long-term storage and archiving of urine samples followed by storing them dry in a vacuum pouch (Gao 2013).

Tears

The phosphate buffer saline containing the test strip is stored at – 80 °C with proper labeling.

Fig. 4 Collection of tears

Handling of biological fluids

Use of medical gloves and masks at all stages of procurement and handling of biological fluids is a mandatory requirement for the purposes of personal safety and sample integrity.

Biological samples require to be thawed before use in any assay or omics-based strategies. The thawing of such samples has a recommended method. The samples are taken out of − 20 °C or − 80 °C refrigerators. Use of cryo gloves is recommended. They are immediately transferred to ice boxes and left to thaw. Till they reach liquid state without any trace of frozen material in the tubes, they are not to be disturbed or used further. Once in the fluid state, they are ready for use. Even then, care should be taken that they are always embedded in ice with minimal holding of tubes in hands and the remaining sample after use should be refrigerated immediately. Sterile pipette tips should be used for each sample. Mixing of samples or their contamination due to use of improper use of pipette tips should be avoided.

Biological fluids for translational studies resulting in potential protein markers

Blood plasma/serum

Biological fluids, particularly blood, has been a subject of fascination among laymen, clinicians and biomedical scientists long before these words came into formal terminology. An example would be the word bloodline, connotating that blood of the father conferred certain characteristics to the offspring, gaining prime importance among the nobles and autocrats in medieval times. Although now known to be a misnomer after the advent of mendelian genetics, it is still in use in non-scientific parlays. In case of other medical contingencies though, biological fluids, particularly blood, gained attraction as more and more biological phenomena were unraveled, particularly in diagnostic capacities. In case of many infectious diseases, like malaria, blood of the patient is of prime importance as it can be used for correct diagnosis of disease. In conditions like HIV/AIDS, blood of the patient is paramount as the gold standard diagnostic sample. Tests like complete blood count have become a ubiquitous preliminary test for screening multiple conditions from leukemia to anemia. Other clinical blood tests, like folate, cholesterol, glucose tests are routinely administered and accepted as the optimal tests for specific conditions. Conditions like damaged heart muscle, elevated inflammation and clotting problems

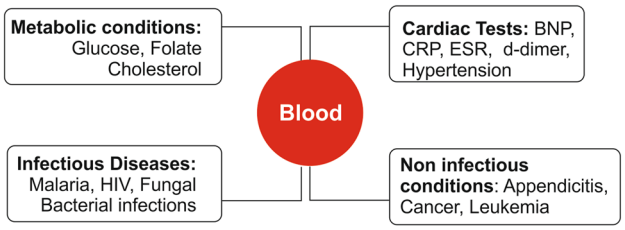

Fig. 5 Uses of blood in diagnosis of various ailments and conditions

are also detected via blood tests like cardiac enzyme (troponin) test, BNP test, CRP test, ESR test and d-dimer tests (Fig. 5). Thus, for the major part of the past 50 years blood has gained more and more importance clinically as a sample of choice. Some highly informative reviews, mostly by Anderson, for basic understanding of plasma proteome and its journey as a diagnostic sample of choice are cited for the interested readers (Anderson and Anderson 2003; Anderson 2010; Anderson et al. 2004; Zhang et al. 2010; Nanjappa et al. 2013).

In case of translational biomedical research, this gain in prominence of blood as a sample of diagnostic value has been consolidated. As diagnosis moves more and more towards molecular tests, omics-based strategies and targeted molecules, blood has provided more and more insights of diagnostic value in multiple domains (Birgisson et al. 2018; Heimburger et al. 2000). Blood has been implicated in diagnosis of invasive aspergillosis (Mengoli et al. 2009; Kami et al. 2001), non-invasive prenatal diagnosis of fetal genetic status (Wright and Burton 2008), resistant hypertension (Calhoun et al. 2008), meningococcal disease (Newcombe et al. 1996), visceral leishmaniasis (Antinori et al. 2007), bacteremia (Wellinghausen et al. 2009) and child appendicitis (Wang et al. 2007).Cancerous tissue is well reported to be hypoxic and the hypoxic environment has been associated with clinical outcomes and targeted therapy (Brown and Wilson 2004; Wilson and Hay 2011; Vaupel and Mayer 2007). Brown, Wilson and Hay opined in two beautiful reviews that hypoxia, being an integral feature of cancer, also provided a context for targeted therapy. They suggested that HIF-1 and other hypoxia responsive genes/proteins (aiding cell survival during hypoxia) provided an avenue for targeted therapy against invasive metastatic cancer cells that were resistant to both chemotherapy and radiotherapy (Brown and Wilson 2004; Wilson and Hay 2011). So much is the importance of blood that entire articles have been dedicated to its proteomic profiling in context of cancer (Omenn 2006). Multiple articles have found important clues in the plasma proteome for various types of cancer (Hanash et al. 2008; Kakisaka et al. 2007; Honda et al. 2005; Cheng et al. 2005; Leth-Larsen et al. 2010; Pan et al. 2011; Gautam et al. 2012) with some articles also including microRNAs (Wang et al.

2009; Lo et al. 2012) in this category and others linking the changes in animal models and humans for translational accuracy (Faca et al. 2008). In the context of hypobaric hypoxia related molecular and omics studies, particularly proteomics, blood plasma and serum have again been of exceptional value due to their diagnostic value as well as elucidation of the molecular response to hypobaric hypoxia. After HIF-1 being established as the main cog in the hypobaric hypoxia responsive protein network (Hochachka and Rupert 2003; Engebretsen et al. 2007; Maxwell 2005; Semenza 2004), the search for prognostic markers against high-altitude illnesses has held the fancy of researchers for long. To this end, multiple scattered plasma proteins have been identified and implicated in patients suffering from AMS, HACE and HAPE.

Acute mountain sickness (AMS) was initially thought of only as a collection of symptoms like headache, nausea, vomiting and insomnia, brought about by antidiuresis at altitude (Johnson and Rock 1988). AMS was also believed to be a pre-disposing factor to high altitude cerebral edema (HACE). Later on, with multiple previous studies on interrelated aspects like peripheral edema, chest rales and blood brain barrier (BBB) bearing no significant fruit (Roach et al. 2000; Gertsch et al. 2004; Roach and Hackett 2001; Bartsch et al. 2004; Singh et al. 1969), in the early 2000 s, researchers observed that AMS is indeed a vasogenic edema but with causal factors rooted in redox homeostasis mechanisms of the central nervous system (Bartsch et al. 2004; Bailey et al. 2009a, b, c). This makes blood plasma the prime tool to assess AMS in mountaineers. Although it is clear that redox homeostasis is essential to unfolding the pathophysiology of AMS, some authors rebut the assumption that strengthened antioxidant processes will prevent AMS (Julian et al. 2013). Another study has decoupled BBB disruption from AMS and reported that cerebral autoregulation and altered redox metabolism are the factors associated with AMS (Bailey et al. 2009a, b, c). Bartsch et al. had reported in the late 80 s that atrial natriuretic peptides may be involved in AMS based on failure of fluid homeostasis at high altitude (Bartsch et al. 1988). Bartsch and colleagues further reported the increased aldosterone and vasopressin (both proteins are involved in fluid homeostasis) levels prior to incidence of AMS in individuals exposed to high altitude (Bartsch et al. 1991). Another study by Bartsch and colleagues which showed interlinks between AMS and HAPE also reported the possibility of blood coagulation factors being used as biomarkers of both AMS and HAPE (Bartsch et al. 1989). A more recent study by Lu et al. stated that plasma proteins involved in TCA cycle can be potential biomarkers for differentiating between AMS resistant and AMS susceptible individuals. The authors further stated that AMS susceptibility may depend on the inability of certain individuals to reduce oxygen consumption by repressing TCA cycle at high altitude (Lu et al. 2018). NO (nitric oxide) bioavailability has also been linked to AMS. Bailey and colleagues, using jugular vein and radial artery blood samples from ten men, had observed that acute passive hypoxia (12.9% O_2 for 9 h) caused increased 3-nitrotyrosine levels and decreased nitrite levels in plasma which positively correlated with AMS scores (Bailey et al. 2009a, b, c). VEGF and VEGF receptor 1 levels in the plasma have also been implicated in AMS by van Patot and co-workers (van Patot et al. 2005). They observed that in AMS patients there is higher free VEGF and lesser soluble VEGF receptor 1 as compared to controls. Based on these evidences, AMS has revealed an extensive plasma proteome footprint that may not only help diagnosis but also elucidate to an extent the molecular processes affected by AMS (Fig. 6).

High altitude cerebral edema (HACE), being the end-stage for AMS, in the early 1900 s was treated as an edema of the brain observed upon visiting high altitude areas. Symptoms are very severe including altered consciousness and ataxia. AMS is considered an important milestone in the journey towards HACE. Another important factor, although contested, is that HACE usually affects a majority of people already suffering from either AMS or high-altitude pulmonary edema (HAPE) (Chawla 2009). Interestingly, till date, HACE has no known plasma proteins associated with it. This may stem from the advances in MRI and other medical procedures as well as the clear and unmistakable symptoms of the disease (beginning with AMS). Also, the previous view that blood plasma may not harbor markers of cerebral stress may have contributed to a lack of investigations in this direction. However, the recent knowledge pertaining to a "glymphatic system" in central nervous system controlling not only metabolite but also tissue fluid homeostasis in the brain (Benveniste et al. 2017) as well as its critical role in traumatic brain injury (Plog et al. 2015) pave the way for a fresh set of investigations into blood based proteins that can predict chances of HACE at altitude (Fig. 6). The glymphatic system is a newly discovered system of perivascular tunnels comprising astroglial cells that is active during sleep and

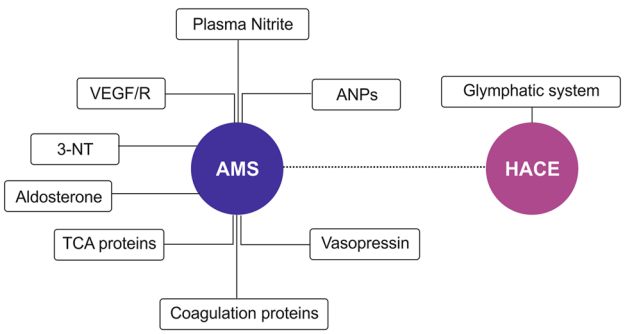

Fig. 6 Reported protein markers for AMS in blood plasma

functions in removing macroscopic waste from the central nervous system (Jessen et al. 2015).

The third prong of the trident of high altitude illnesses, high altitude pulmonary edema (HAPE) is a form of non-cardiogenic pulmonary edema where the lack of alveolar fluid clearance and disruptive vasculo-endothelial diffusion pressures cause a build-up of fluids, inflammatory in nature, in the lungs (Scherrer et al. 2010). Symptoms include pink frothy sputum, labored breathing and dyspnea at rest. Like HACE, HAPE is also a life-threatening condition and requires immediate medical attention. HAPE is the most common cause of death at high altitude (Basnyat 2005; Basnyat and Murdoch 2003). HAPE has received equal, if not more, attention when compared to HACE. Like all high-altitude illnesses, HAPE also requires administration of oxygen, descent of at least 500 m and rest on subsequent days. Multiple plasma studies have been conducted on HAPE patients. Initially, genomic variations were thought to be the culprits for differential response of different subjects to similar altitude. Endothelin-1 (ET-1) was one of the first genes implicated in the occurrence of HAPE (Droma et al. 1996; Sartori et al. 1999). ET-1 gene variants have also been associated with adaptation to hypobaric hypoxia in natives residing at high altitude (Rajput et al. 2006). In 2006, another related gene, angiotensin converting enzyme, along with ET-1 was reported to have certain allelic combinations that makes the individual susceptible to HAPE (Charu et al. 2006). However, with further progress, the onus shifted from genomic to proteomic/metabolomic investigations. This is because high altitude illnesses are expressed physiologically only till the individual is subjected to high altitude. Upon timely descent, no symptoms or irreversible physiological damage is observed. Such dynamic behavior is mirrored more aptly by proteome and metabolome of subjects. Transpulmonary ET-1 plasma levels along with plasma nitrite levels were reported to be associated with pulmonary artery vascular tone and subsequent pulmonary hypertension (Berger et al. 2009). This provides a more direct mechanistic evidence of involvement of ET-1 in HAPE rather than empirical associations between a certain allele and a cohort of subjects previously affected by concerned disease. A recent study by Barker et al. reported that AMS and HAPE had common plasma protein markers like angiopoietin like 4 protein and resistin. They further reported that plasma ET-1 and soluble kinase domain receptor protein were elevated only in cases of HAPE. Also, corin and angiotensin converting enzyme plasma levels decreased during HAPE. These proteins also correlated with physiological measures like SpO_2 and blood counts (Barker et al. 2016). Studies have also been conducted from our lab regarding plasma proteomics of HAPE patients. Ahmad et al. had identified Apolipoprotein A1 and haptoglobin as prospective biomarkers for HAPE (Ahmad et al. 2011). Afterwards they elucidated sulfotransferase 1A1 as a marker for HAPE (Ahmad et al. 2015). Zhang et al. reported serum levels of haptoglobin, alpha-1-anti trypsin, C3 and apolipoproteins A1 and A4 to be differentially expressed in HAPE patients as compared to normal subjects (Zhang et al. 2013). Boos et al. have reported that markers of cardiac function like brain natriuretic peptide (BNP) and high-sensitivity cardiac troponin T(hs-cTnT) associate strongly with pulmonary artery pressure and HAPE, thus making them suitable markers for the same (Fig. 7). However, the authors suggest similarity of increased BNP and hs-cTnT plasma levels with those observed in genuine heart failure and myocardial infarction (Boos et al. 2013). Gupta et al. also corroborate BNP levels as predictive of HAPE susceptibility (Gupta et al. 2016). However, the sample size is far too small, the p value threshold (0.05) too generalized and choice of subjects (individuals treated for HAPE many months ago) too vague for any definitive conclusions. This is further compounded by the average AUC value obtained in ROC curve. But in light of other similar results (Mellor et al. 2014), the conclusions are justified. Another recent report describes inflammatory signaling based marker assessment for HAPE. In this study, the authors report CRP, IL-6 and soluble urokinase-type plasminogen activator receptor (suPAR) to be capable of assessing susceptibility to HAPE. However, this study also suffers from choice of subjects (individuals who had suffered HAPE in the past). Nonetheless, suPAR is reported in this study to be capable of assessing HAPE susceptibility independently prior to high altitude ascent (Hilty et al. 2016). Sikri and Bhattachar have provided hard hitting commentary on this study, highlighting the insignificant differences in suPAR levels between control and HAPE susceptible population as well as the convoluted association between AMS, HAPE and dexamethasone reported in the above mentioned study (Sikri and Bhattachar 2017). Thus, although we have found many interesting potential candidates for assessment of susceptibility and prediction of high altitude illnesses via use of blood-based proteins, our lack of integrative/collaborative research methodologies, intrinsically low availability of patient samples and focus towards unilateral protein markers

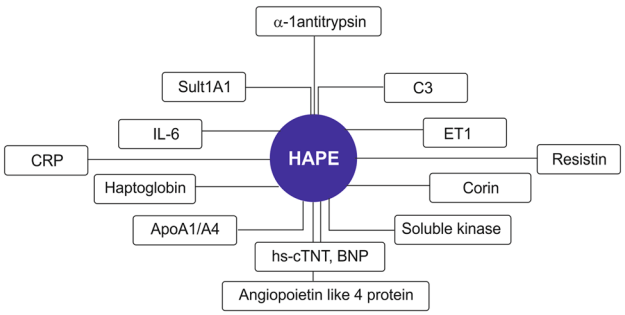

Fig. 7 Reported protein markers of HAPE in blood plasma

instead of marker protein panels has led to slow progress and elusive clinical utility.

This problem is further aggravated by limited studies on assessing healthy individuals at high altitude. Previous estimates suggested that about 25 percent of Colorado skiers, 50 percent of Himalayas trekkers, and nearly 85 percent of those who fly directly to the Mount Everest region suffer altitude induced illnesses, mostly AMS upon exposure (Basnyat and Murdoch 2003; Murdoch 1995; Honigman et al. 1993; Hackett et al. 1976). Maggiorini et al. studied the altitude dependent incidence of AMS in the Swiss Alps. They stated the incidence of AMS to be 9% at 2850 m, 13% at 3050 m, 34% at 3650 m, and 53% at 4559 m, revealing an increase with altitude (Maggiorini et al. 1990). Thus, it's observed that the more populous relatively lower high altitude areas tend to have lower incidence rates of high altitude illnesses. As a corollary, these places also have the greater number of healthy mountaineers/tourists/trekkers. Extreme regions like the Mount Everest are the destination of only a handful even though they have higher incidence rates of high altitude illnesses. So most of the high altitude sojourners are healthy ones and thus more studies detailing acclimatization to altitude in terms of plasma proteome based markers should have been the norm. This would have helped establish two inter-related facets of high altitude exposure. The first one is the normal "healthy" response to hypobaric hypoxia which leads to immediate acclimatization. The second facet deals with long-term acclimatization and adaptation of those individuals who were born native to high altitude conditions. The proteins and their quantification trends in the healthy individuals not only serve as a baseline measure against the abnormal protein expression observed in those afflicted by high altitude illnesses but also link to the broader spectrum of diseases like cancer and diabetes. In a study authored by Siervo and colleagues, chronic exposure to high altitude was linked to insulin resistance and subsequent inflammation and oxidative stress in acclimatized individuals (Siervo et al. 2014). Multiple studies havestated hyperbaric oxygen therapy as a treatment strategy particularly for metastatic cancers (Daruwalla and Christophi 2006; Poff et al. 2013), indicating deep molecular signaling redundancies in cancer and hypobaric hypoxia. Both are known to up-regulate glycolytic processes while moving the cells away from OXPHOS (oxidative phosphorylation) pathways (Liberti and Locasale 2016; Connett et al. 1990). Lu and co-workers have previously elucidated the role of aerobic glycolysis in activating HIF-1a, a well-known master regulator of hypobaric hypoxia induced proteome response, in carcinogenesis (Lu et al. 2002). This and many other articles comprise observations and findings of the 2007 Caudwell Xtreme Everest Expedition which was designed to include only those who had previous event free ascents (Grocott et al. 2010; Levett et al. 2010). Plasma biomarkers were stated as one of the main outcome measures (Levett et al. 2010). Multiple plasma proteins were observed to be potential markers of many different molecular/physiological events occurring at high altitude. It was observed that erythropoietin, 8-isoprostanes, guanosine 3′, 5′-cyclic monophosphate and nitrite levels in plasma strongly correlate with pulmonary artery pressure after progressive ascent (2 weeks) to 5300 m (Luks et al. 2017). Another study revealed that acclimatization to altitude is depicted by increased NO bioavailability via increased NO production and cGMP activity and initial consumption of S-nitrosothiols (Levett et al. 2011). Fago and colleagues have reviewed the integrated nature of NO and H_2S signaling pathways during the hypoxic response in the plasma proteome. They emphasize the need for identifying the linkers between NO, H_2S and hypoxia (Fago et al. 2012). In a very recent study, Cumpstey et al. reported the personalized and dynamic nature of blood thiol and NO arteriovenous gradients occurring in humans during altitude exposure which may be later used for personalized redox therapeutics (Cumpstey et al. 2019) (Fig. 8).

Assessment of plasma proteins in humans exposed to high altitude is a very old and effective approach, with old studies suggesting increased hematocrit, plasma creatinine and transferrin levels in acclimatized individuals (Rennie et al. 1972; Becker et al. 1957). Diverging a bit, in a more recent study by Yasmin and colleagues, it was observed that in high altitude natives transferrin levels were lower as compared to sea level residents (Ahmad et al. 2013). This may be indicative of fundamental differences in acclimatization and adaptation in terms of protein expression and signaling networks. Hartmann and co-workers reported important cytokines like C-reactive protein, interleukin-6 and interleukin-1 receptor antagonist to have increased plasma concentrations during high altitude hypoxia exposure in otherwise healthy mountaineers (Hartmann et al. 2000). This is in contrast to a previous study suggesting cytokine levels, particularly suPAR, to be an indicative of HAPE susceptibility (Hilty et al. 2016). Involvement of erythrocyte proteins in maintaining acid–base balance was also reported in a

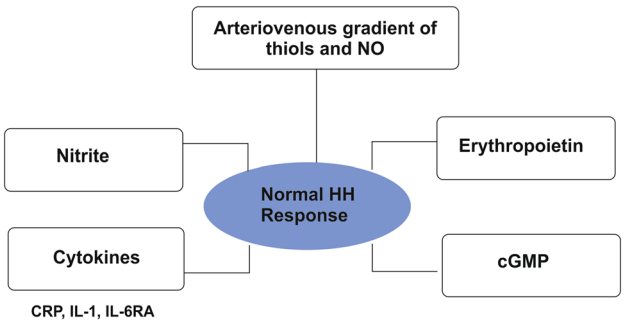

Fig. 8 Reported proteins implicated in acclimatization and healthy hypobaric hypoxia (HH) response

similar study by Juel et al. (Juel et al. 2003). Multiple other studies regarding high altitude exposure have also been performed by the author's institution. These studies detail the plasma proteome expression of both natives and low landers at high altitudes. Ahmad and colleagues uncovered a fine anti-inflammatory axis in high altitude natives that controls inflammation (Ahmad et al. 2013). Although high altitude natives differ in their adaptation mechanisms both at physiological and molecular levels (Beall 2007; Beall et al. 1997; Moore 2001; Beall et al. 1998; Beall 2006; Xing et al. 2008), they offer an overview of the best organismal strategies to control the adverse effects of high altitude hypoxia. However, one must keep in mind that most of these alterations in adapted natives is a result of genetic differences. Other studies by the same author explored the effects of simulated altitude exposure in SD rats (Ahmad et al. 2014) and then translated them into human HAPE patients (Ahmad et al. 2015), while also observing the redox effects of simulated altitude exposure. Tyagi et al. observed that calpain and other platelet proteins have altered activity during high altitude exposure thus increasing chances of thrombosis (Tyagi et al. 2014). Thrombosis as a consequence of NLRP3 inflammasome activation during hypoxia was reported by Gupta and colleagues very recently. Bradykinin, a known inflammatory protein associated with the coagulation system was also highlighted during hypoxia, particularly in NO signaling (Hofman et al. 2016). Padhy and colleagues observed an increased production of NO in low land travelers and more prominently in Ladhaki high altitude natives (during high altitude exposure) as a consequence of modulation of eNOS activity via kininogen-kallikrein-bradykinin, again implicating NO in both acclimatization and adaptation to altitude (Padhy et al. 2016a, b). In a previous report, eNOS gene had been implicated in the adaptation of Sherpas to Tibetan highlands (Droma et al. 2006). Thus, the proteins involved in thrombosis, inflammation and NO signaling are interlinked and may provide benefits upon hypoxia exposure if modulated correctly. As mentioned before in this review, NO is also linked to H_2S signaling in context of hypoxia (Fago et al. 2012). A preliminary study by Kumar and co-workers in rat model speculated that augmenting H_2S levels in brain may have beneficial effects during high altitude exposure (Kumar et al. 2016). By analysis of the homologous protein networks between rat and human during hypobaric hypoxia exposure using an in silico hybrid (Paul et al. 2017), the above speculation appears plausible. Thus, common/linked proteins between NO and H_2S signaling may open new dimensions in the search for effective interventions and reliable markers for high altitude acclimatization. Overall, from all available literature, proteins implicated in redox homeostasis, energy homeostasis, fluid homeostasis, acute phase response signaling, coagulation system, complement system and NO-H_2S signaling seem to be highlighted repeatedly by investigations targeting the plasma proteome of individuals exposed to high altitude hypoxia (Fig. 8).

Newer strategies include study of saliva and other biofluids, that are truly non-invasive in nature. However, despite their distinct ease of collection and clinical use as a diagnostic sample, one major drawback is that they must be complemented with greater understanding of the underlying global molecular events. This is due to the fact that except blood, all other biofluids provide no significant molecular insight. Thus, as we move on to newer vistas for exploration and improvement, one must not leave the on-going works incomplete.

Saliva

Another biological fluid, saliva, being non-invasive in nature has enormous diagnostic potential. Recently, it has gained evident attention as an effective strategy for screening, diagnosis, prognosis and monitoring post-therapy status due to its easy sample collection and processing, less chances of contracting infections, low cost and better tolerance by patients (Schafer et al. 2014; Yoshizawa et al. 2013; Chiappin et al. 2007; Kaczor-Urbanowicz et al. 2017; Lee and Wong 2009; Pfaffe et al. 2011). Translational research from the previous decades suggested that chronic disease such as cancer, diabetes, cardiovascular, neurological and pulmonary diseases are associated with continued oxidative stress (Reuter et al. 2010). In translational research, saliva has been explored in the detection of oral cancer (de Jong et al. 2010; Hu et al. 2007a, b; Gallo et al. 2016), Sjogren's syndrome (Giusti et al. 2007; Hu et al. 2007a, b; Peluso et al. 2007), breast cancer (Streckfus et al. 2008), lung cancer (Xiao, H., et al., Proteomic analysis of human saliva from lung cancer patients using two-dimensional difference gel electrophoresis and mass spectrometry. Mol Cell Proteomics 2012) and systemic disorders such as hepatitis, HIV and HCV (Elsana

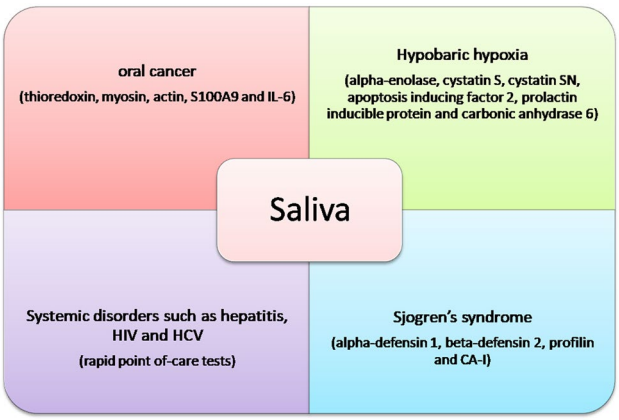

Fig. 9 Saliva in diagnosis of various ailments and the identified biomarker candidates

et al. 1998; Yaari et al. 2006; Hodinka et al. 1998) (Fig. 9). An initial study by Shen Hu et al. gave a proof of concept for exploring salivary proteins in oral cancer by revealing thioredoxin as a salivary biomarker for human oral cancer (Hu et al. 2007a, b). Another study by Ebbing P. de Jong et al. revealed myosin and actin as promising salivary biomarkers for distinguishing pre-malignant and malignant oral lesions (de Jong et al. 2010). A recent study by Eva Csosz et al. investigated oral squamous cell carcinoma (OSCC) biomarkers in a Hungarian population and highlighted the importance of identification of population tailored biomarkers. In this study, S100A9 and IL-6 were shown to be candidate biomarkers for OSCC (Csosz et al. 2017). In Sjogren's syndrome (SS), a study by G. Peluso et al. on saliva from patients with primary SS revealed higher levels of alpha-defensin 1 and the presence of beta-defensin 2 could be markers of oral inflammation in SS patients group (Peluso et al. 2007). Another study by Omer Deutsch et al. identified profilin and CA-I as biomarker candidates for Sjögren's syndrome following high-abundance protein depletion (Deutsch et al. 2015). In cases of non-oral cancers such as breast and lung cancers, various researchers suggested modifications in the salivary proteome and provided proof of concept for candidate biomarkers (Streckfus et al. 2008; Xiao et al. 2012; Bigler et al. 2009; Streckfus and Bigler 2016). In HIV and HCV, rapid point of-care HIV tests utilize oral fluids to rapidly provide test results to patients (Hodinka et al. 1998; Fernandez Rodriguez et al. 1994). Another example is its use for determining hormone levels, including estradiol, progesterone and testosterone, DHEA, and cortisol (Groschl 2008). In the context of hypobaric hypoxia related events, saliva has been studied due to its diagnostic potential. An initial study, in 1990s, suggested an increased salivary flow rate and low potassium concentration in response to acute hypobaric hypoxia exposure (Pilardeau et al. 1990). Another study done by McLean reported decrease in aldosterone response to both renin-angiotensin and ACTH stimulation at high altitude (McLean et al. 1989). Additionally, there is a rise in the salivary activities of aminotransferases during HH exposure (Mominzadeh et al. 2014). Another researcher, Woods DR et al. recently reported an alteration in the evidently related molecule, salivary cortisol and suggested an elevated cortisol may contribute to fluid retention associated with acute mountain sickness (Woods et al. 2012).

In omics-based studies, particularly proteomics, only a handful of studies have been performed so far. Jain et al. have reported significantly altered proteins such as alpha-enolase, cystatin S, cystatin SN, apoptosis inducing factor 2, prolactin inducible protein and carbonic anhydrase 6 and plausible pathways involving these proteins such as impaired glycolysis, inflammation and respiratory alkalosis during HH exposure(Jain et al. 2018). There is an enormous requirement of saliva based proteomic studies for the screening of molecular events occurring after HH exposure due to fewer studies.

Urine

Urine, another non-invasive biological fluid, is highly desirable for biomarker analysis as it can be collected in relatively large volumes. Urine, being a filtrate of the blood, accommodates the modifications in the internal environment to a higher degree and those changes are more likely to be detectable in urine in higher magnitudes than their counterparts in blood. Urinary proteome contains a variety of potential biomarkers for overall health and organ related pathophysiological conditions as nearly 30% of the urinary proteome is derived from glomerular plasma filtration and 70% of the urinary proteome originates from the urogenital tract (Harpole et al. 2016; Decramer et al. 2008). Therefore, urinary proteomics is perfect diagnostic research in assessment of disease risk and mechanisms; and predicting optimal therapy (Collins and Varmus 2015). In translational research, urine proteome has been explored in variety of non-kidney associated diseases including acute appendicitis (Kentsis et al. 2010; Kharbanda et al. 2012), infectious diseases such as Tuberculosis (Young et al. 2014), cancer (Frantzi et al. 2015; Raimondo et al. 2014), cardiovascular disease (Brown et al. 2015; Zhang et al. 2015a, b), and aging (Nkuipou-Kenfack et al. 2015) (Fig. 10). In oxidative stress related manifestations such as cancer and aging, scientists initially compared different techniques for urinary proteome and peptidome of renal cell carcinoma patients followed by identification of PCK1 and SNRPF as biomarkers for renal

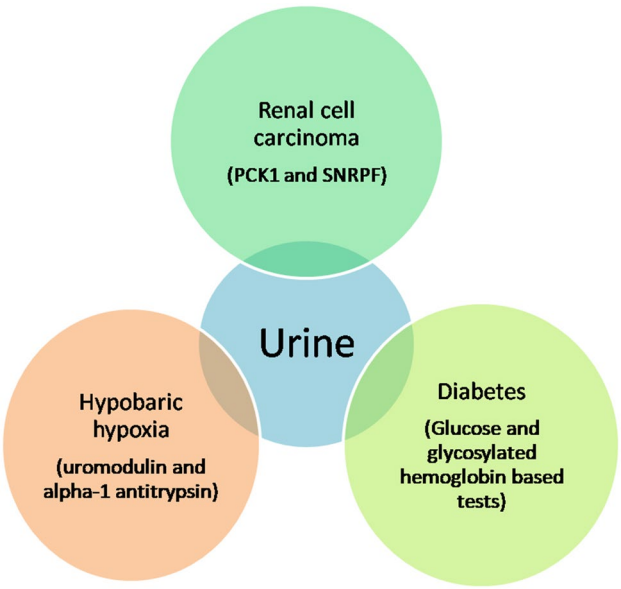

Fig. 10 Urine in diagnosis of various ailments and the identified biomarker candidates

cell carcinoma (Frantzi et al. 2015; Raimondo et al. 2014; Schiffer et al. 2012; Chinello et al. 2016; Sun et al. 2016). And, Nkuipou-Kenfack et al. reported perturbations in collagen homeostasis, trafficking of toll-like receptors and endosomal pathways, degradation of insulin-like growth factor-binding proteins in pathological ageing (Nkuipou-Kenfack et al. 2015). For diagnosis of diabetes, urine based glucose tests have been developed and tests based on glycosylated hemoglobin are in development (Pleitez et al. 2012; Zhang et al. 2015a, b). In hypobaric hypoxia, Mainini et al. reported that urinary proteome is modified upon HH exposure. They have identified six modulated peptides during HH exposure, two of them are the fragments of glycoprotein uromodulin and alpha-1 antitrypsin(Mainini et al. 2012). Alpha-1 antitrypsin is previously being studied in plasma and found to be modulated during HH exposure(Zhang et al. 2013). The presence of similar modulated proteins in urine and plasma at high altitude suggests the scope of urinary proteomics at high altitude.

Tears

Another important non-invasive biological fluid is tears. Tears consist of secretions containing thousands of biomolecules such as proteins/peptides, lipids, electrolytes and metabolites from lacrimal gland, goblet cells, cornea and vascular sources (Zhou and Beuerman 2012). Although, tears are collected in small volume, it offers various advantages such as non-invasive sampling using Schirmer's strips, easy collection and closeness to the eye-disease site. Also, sample preparation is direct as depletion of abundant proteins such as albumin is not required in tear proteome analysis, thus, results in high quality proteome coverage (Zhou and Beuerman 2017). In translational research, tears are explored in the ocular diseases such as dry eye disease (Aluru et al. 2012; Choi et al. 2012; Grus et al. 2005; Enriquez-de-Salamanca et al. 2010; Zhou et al. 2009), Sjogren's syndrome (Aqrawi et al. 2017), thyroid associated orbitopathy (Huang et al. 2014), and glaucoma (Pieragostino et al. 2012); and systemic diseases such asdiabetic retinopathy(Costagliola et al. 2013; Csosz et al. 2012; Kim et al. 2012; Torok et al. 2015), cancer(Evans et al. 2001) and Parkinson's disease(Borger et al. 2015) (Fig. 11). Recently, dry eye disease was reported in association with age mediated oxidative stress (Seen and Tong 2018). Several researchers suggested lacrimal proline rich protein 4 (LPRR4), annexin A1, neutrophil elastase 2, clusterin, apolipoprotein A-II, alpha-enolase, S100 A4, cytokines and chemokines as candidate biomarkers for dry eye disease (Aluru et al. 2012; Choi et al. 2012; Grus et al. 2005; Enriquez-de-Salamanca et al. 2010; Zhou et al. 2009; Yoon et al. 2010; Li et al. 2014). Another study, Lara A. Aqrawi et al. suggested proteins involved in innate immunity (LCN2,

Fig. 11 Tears in diagnosis of various ailments and the identified biomarker candidates

SIRPA and LSP1), cell signalling (CALM), wound repair (GRN and CALML5), adipocyte differentiation (APMAP), TNF-α signalling (CPNE1) and B cell survival (PRDX3) can serve as diagnostic markers for SS(Aqrawi et al. 2017). In systemic disease (diabetic retinopathy), researchers suggested combined methods for its screening using retina photographs and tear proteomics; and TNF-alpha, lipocalin 1, lactotransferrin, lacritin, lysozyme C, lipophilin A, immunoglobulin lambda chain, LCN-1, HSP27 and B2 M as markers for early diagnostics of diabetic retinopathy(Costagliola et al. 2013; Csosz et al. 2012; Kim et al. 2012; Torok et al. 2015). A study by Evans V et al. reported lacryglobin in human tears as a potential marker for various types of cancer such as cancers of breast, lung, colon, ovary and prostate (Evans et al. 2001).In the context to HH exposure, high altitude has both short and long term exposure effects on eyes. The short-term effects include high-altitude retinopathy, change in corneal thickness, and photokeratitis and long-term effects include pterygium, cataract, and dry eye syndrome (Jha 2012; Gupta et al. 2008). Few studies and surveys reported that hypobaric hypoxia alters tear breakup time and film osmolarity (Jha 2009; Willmann et al. 2014). The lack of proteomic analysis in tears during HH exposure provides ample scope for exploring this amazing biological fluid to the researchers.

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Compliance with ethical standards

Conflict of interest Authors declare no conflict of interest.

References

Ahmad Y et al (2011) Identification of haptoglobin and apolipoprotein AI as biomarkers for high altitude pulmonary edema. Funct Integr Genomics 11(3):407

Ahmad Y et al (2013) An insight into the changes in human plasma proteome on adaptation to hypobaric hypoxia. PLoS One 8(7):e67548

Ahmad Y et al (2014) Proteomic identification of novel differentiation plasma protein markers in hypobaric hypoxia-induced rat model. PLoS One 9(5):e98027

Ahmad Y et al (2015) The proteome of hypobaric induced hypoxic lung: insights from temporal proteomic profiling for biomarker discovery. Sci Rep 5:10681

Aluru SV et al (2012) Lacrimal proline rich 4 (LPRR4) protein in the tear fluid is a potential biomarker of dry eye syndrome. PLoS One 7(12):e51979

Anderson NL (2010) The clinical plasma proteome: a survey of clinical assays for proteins in plasma and serum. Clin Chem 56(2):177–185

Anderson NL, Anderson NG (2003) The human plasma proteome: history, character, and diagnostic prospects. Mol Cell Proteomics 2(1):50

Anderson NL et al (2004) The human plasma proteome: a nonredundant list developed by combination of four separate sources. Mol Cell Proteomics 3(4):311–326

Antezana G et al (1982) Hemodynamic study of high altitude pulmonary edema (12,200 ft). High altitude physiology and medicine. Springer, New York, pp 232–241

Antinori S et al (2007) Clinical use of polymerase chain reaction performed on peripheral blood and bone marrow samples for the diagnosis and monitoring of visceral leishmaniasis in HIV-infected and HIV-uninfected patients: a single-center, 8-year experience in Italy and review of the literature. Clin Infect Dis 44(12):1602–1610

Aqrawi LA et al (2017) Identification of potential saliva and tear biomarkers in primary Sjogren's syndrome, utilising the extraction of extracellular vesicles and proteomics analysis. Arthritis Res Ther 19(1):14

Bailey DM et al (2009a) Emerging concepts in acute mountain sickness and high-altitude cerebral edema: from the molecular to the morphological. Cell Mol Life Sci 66(22):3583–3594

Bailey D et al (2009b) Altered free radical metabolism in acute mountain sickness: implications for dynamic cerebral autoregulation and blood–brain barrier function. J Physiol 587(1):73–85

Bailey DM et al (2009c) Increased cerebral output of free radicals during hypoxia: implications for acute mountain sickness? Am J Physiol Regul Integr Compar Physiol 297(5):R1283–R1292

Barker KR et al (2016) Biomarkers of hypoxia, endothelial and circulatory dysfunction among climbers in Nepal with AMS and HAPE: a prospective case–control study. J Travel Med 23(3). https://doi.org/10.1093/jtm/taw005

Bartsch P et al (1988) Atrial natriuretic peptide in acute mountain sickness. J Appl Physiol 65(5):1929–1937

Bartsch P et al (1989) Coagulation and fibrinolysis in acute mountain sickness and beginning pulmonary edema. J Appl Physiol 66(5):2136–2144

Bartsch P et al (1991) Enhanced exercise-induced rise of aldosterone and vasopressin preceding mountain sickness. J Appl Physiol 71(1):136–143

Bartsch P et al (2004) Acute mountain sickness: controversies and advances. High Alt Med Biol 5(2):110–124

Basnyat B (2005) High altitude cerebral and pulmonary edema. Travel Med Infect Dis 3(4):199–211

Basnyat B, Murdoch DR (2003) High-altitude illness. Lancet 361(9373):1967–1974

Beall CM (2006) Andean, Tibetan, and Ethiopian patterns of adaptation to high-altitude hypoxia. Integr Comp Biol 46(1):18–24

Beall CM (2007) Two routes to functional adaptation: tibetan and Andean high-altitude natives. Proc Natl Acad Sci 104(suppl 1):8655–8660

Beall CM et al (1997) Ventilation and hypoxic ventilatory response of Tibetan and Aymara high altitude natives. Am J Phys Anthropol 104(4):427–447

Beall CM et al (1998) Hemoglobin concentration of high-altitude Tibetans and Bolivian Aymara. Am J Phys Anthropol 106(3):385–400

Becker EL, Schilling JA, Harvey RB (1957) Renal function in man acclimatized to high altitude. J Appl Physiol 10(1):79–80

Benveniste H, Lee H, Volkow ND (2017) The glymphatic pathway: waste removal from the cns via cerebrospinal fluid transport. Neurosci Rev J Bring Neurobiol Neurol Psychiatry 23:454–465

Berger MM et al (2009) Transpulmonary plasma ET-1 and nitrite differences in high altitude pulmonary hypertension. High Alt Med Biol 10(1):17–24

Berne RM (1963) Cardiac nucleotides in hypoxia: possible role in regulation of coronary blood flow. Am J Physiol Legacy Content 204(2):317–322

Bigler LR, Streckfus CF, Dubinsky WP (2009) Salivary biomarkers for the detection of malignant tumors that are remote from the oral cavity. Clin Lab Med 29(1):71–85

Birgisson H et al (2018) Plasma protein profiling reveal osteoprotegerin as a marker of prognostic impact for colorectal cancer. Transl Oncol 11(4):1034–1043

Boos CJ et al (2013) Cardiac biomarkers and high altitude pulmonary edema. Int J Cardiol 167(3):e65–e66

Borger M, Bahr FSM, Grus F, Lingor P (2015) Biomarker sources for Parkinson's disease: time to shed tears? Basal Ganglia 5(2–3):63–69

Brown JM, Wilson WR (2004) Exploiting tumour hypoxia in cancer treatment. Nat Rev Cancer 4(6):437

Brown CE et al (2015) Urinary proteomic biomarkers to predict cardiovascular events. Proteomics Clin Appl 9(5–6):610–617

Calhoun DA et al (2008) Resistant hypertension: diagnosis, evaluation, and treatment: a scientific statement from the American Heart Association Professional Education Committee of the Council for High Blood Pressure Research. Circulation 117(25):e510–e526

Charu R et al (2006) Susceptibility to high altitude pulmonary oedema: role of ACE and ET-1 polymorphisms. Thorax 61(11):1011–1012

Chawla A (2009) Incidence of Acute Mountain Sickness in patients of high altitude pulmonary edema: is the Lake Louise scoring accurate. Ind J Aerospace Med 53(1):1–5

Cheng A-J et al (2005) Oral cancer plasma tumor marker identified with bead-based affinity-fractionated proteomic technology. Clin Chem 51(12):2236–2244

Chiappin S et al (2007) Saliva specimen: a new laboratory tool for diagnostic and basic investigation. Clin Chim Acta 383(1–2):30–40

Chinello C et al (2016) The proteomic landscape of renal tumors. Expert Rev Proteomics 13(12):1103–1120

Choi W et al (2012) Expression of CCR169 and its ligands CCL3, -4, and -5 in the tear film and ocular surface of patients with dry eye disease. Curr Eye Res 37(1):12–17

Collins FS, Varmus H (2015) A new initiative on precision medicine. N Engl J Med 372(9):793–795
Connett R et al (1990) Defining hypoxia: a systems view of VO_2, glycolysis, energetics, and intracellular PO_2. J Appl Physiol 68(3):833–842
Costagliola C et al (2013) TNF-alpha levels in tears: a novel biomarker to assess the degree of diabetic retinopathy. Mediators Inflamm 2013:629529
Csosz E et al (2012) Quantitative analysis of proteins in the tear fluid of patients with diabetic retinopathy. J Proteomics 75(7):2196–2204
Csosz E et al (2017) Proteomics investigation of OSCC-specific salivary biomarkers in a Hungarian population highlights the importance of identification of population-tailored biomarkers. PLoS One 12(5):e0177282
Cumpstey AF et al (2019) Pushing arterial-venous plasma biomarkers to new heights: a model for personalised redox metabolomics? Redox Biol 21:101113
Daruwalla J, Christophi C (2006) Hyperbaric oxygen therapy for malignancy: a review. World J Surg 30(12):2112
de Jong EP et al (2010) Quantitative proteomics reveals myosin and actin as promising saliva biomarkers for distinguishing premalignant and malignant oral lesions. PLoS One 5(6):e11148
Decramer S et al (2008) Urine in clinical proteomics. Mol Cell Proteomics 7(10):1850–1862
Deutsch O et al (2015) Identification of Sjogren's syndrome oral fluid biomarker candidates following high-abundance protein depletion. Rheumatology (Oxford) 54(5):884–890
Droma Y et al (1996) Endothelin-1 and interleukin-8 in high altitude pulmonary oedema. Eur Respir J 9(9):1947–1949
Droma Y et al (2006) Genetic contribution of the endothelial nitric oxide synthase gene to high altitude adaptation in sherpas. High Alt Med Biol 7(3):209–220
Elsana S et al (1998) HCV antibodies in saliva and urine. J Med Virol 55(1):24–27
Engebretsen BJ et al (2007) Acute hypobaric hypoxia (5486 m) induces greater pulmonary HIF-1 activation in hilltop compared to madison rats. High Alt Med Biol 8(4):312–321
Enriquez-de-Salamanca A et al (2010) Tear cytokine and chemokine analysis and clinical correlations in evaporative-type dry eye disease. Mol Vis 16:862–873
Evans V et al (2001) Lacryglobin in human tears, a potential marker for cancer. Clin Exp Ophthalmol 29(3):161–163
Faca VM et al (2008) A mouse to human search for plasma proteome changes associated with pancreatic tumor development. PLoS Med 5(6):e123
Fago A et al (2012) Integrating nitric oxide, nitrite and hydrogen sulfide signaling in the physiological adaptations to hypoxia: a comparative approach. Comp Biochem Physiol A: Mol Integr Physiol 162(1):1–6
Fernandez Rodriguez E et al (1994) Detection of HIV antibodies in saliva using a rapid diagnostic immunoenzyme assay. Rev Clin Esp 194(7):523–525
Frantzi M et al (2015) Recent progress in urinary proteome analysis for prostate cancer diagnosis and management. Expert Rev Mol Diagn 15(12):1539–1554
Gallo C et al (2016) Potential salivary proteomic markers of oral squamous cell carcinoma. Cancer Genom Proteomics 13(1):55–61
Gao Y (2013) Urine—an untapped goldmine for biomarker discovery? Sci China Life Sci 56(12):1145–1146. https://doi.org/10.1007/s11427-013-4574-1
Gautam P et al (2012) Proteins with altered levels in plasma from glioblastoma patients as revealed by iTRAQ-based quantitative proteomic analysis. PLoS One 7(9):e46153
Gertsch JH et al (2004) Randomised, double blind, placebo controlled comparison of ginkgo biloba and acetazolamide for prevention of acute mountain sickness among Himalayan trekkers: the prevention of high altitude illness trial (PHAIT). BMJ 328(7443):797
Giusti L et al (2007) Proteome analysis of whole saliva: a new tool for rheumatic diseases—the example of Sjogren's syndrome. Proteomics 7(10):1634–1643
Grocott MP et al (2010) Caudwell xtreme Everest expedition. High Alt Med Biol 11(2):133–137
Groschl M (2008) Current status of salivary hormone analysis. Clin Chem 54(11):1759–1769
Grus FH et al (2005) SELDI-TOF-MS ProteinChip array profiling of tears from patients with dry eye. Invest Ophthalmol Vis Sci 46(3):863–876
Gupta N et al (2008) Prevalence of dry eye at high altitude: a case controlled comparative study. High Alt Med Biol 9(4):327–334
Gupta RK et al (2016) Elevated pulmonary artery pressure and brain natriuretic peptide in high altitude pulmonary edema susceptible non-mountaineers. Sci Rep 6:21357
Hackett P, Rennie D, Levine H (1976) The incidence, importance, and prophylaxis of acute mountain sickness. Lancet 308(7996):1149–1155
Hanash SM, Pitteri SJ, Faca VM (2008) Mining the plasma proteome for cancer biomarkers. Nature 452(7187):571
Harpole M, Davis J, Espina V (2016) Current state of the art for enhancing urine biomarker discovery. Expert Rev Proteomics 13(6):609–626
Hartmann G et al (2000) High altitude increases circulating interleukin-6, interleukin-1 receptor antagonist and C-reactive protein. Cytokine 12(3):246–252
Havanapan PO, Thongboonkerd V (2009) Are protease inhibitors required for gel-based proteomics of kidney and urine? J Proteome Res 8(6):3109–3117
Heimburger O et al (2000) Hand-grip muscle strength, lean body mass, and plasma proteins as markers of nutritional status in patients with chronic renal failure close to start of dialysis therapy. Am J Kidney Dis 36(6):1213–1225
Henson BS, Wong DT (2010) Collection, storage, and processing of saliva samples for downstream molecular applications. Methods Mol Biol 666:21–30
Hilty MP et al (2016) Soluble urokinase-type plasminogen activator receptor plasma concentration may predict susceptibility to high altitude pulmonary edema. Mediat Inflamm 2016:1942460. https://doi.org/10.1155/2016/1942460
Hochachka PW, Rupert JL (2003) Fine tuning the HIF-1 'global'O2 sensor for hypobaric hypoxia in Andean high-altitude natives. BioEssays 25(5):515–519
Hodinka RL, Nagashunmugam T, Malamud D (1998) Detection of human immunodeficiency virus antibodies in oral fluids. Clin Diagn Lab Immunol 5(4):419–426
Hofman Z et al (2016) Bradykinin: inflammatory product of the coagulation system. Clin Rev Allergy Immunol 51(2):152–161
Honda K et al (2005) Possible detection of pancreatic cancer by plasma protein profiling. Can Res 65(22):10613–10622
Honigman B et al (1993) Acute mountain sickness in a general tourist population at moderate altitudes. Ann Intern Med 118(8):587–592
Hu S et al (2007a) Discovery of oral fluid biomarkers for human oral cancer by mass spectrometry. Cancer Genomics Proteomics 4(2):55–64
Hu S et al (2007b) Salivary proteomic and genomic biomarkers for primary Sjogren's syndrome. Arthritis Rheum 56(11):3588–3600
Huang D et al (2014) Changes of lacrimal gland and tear inflammatory cytokines in thyroid-associated ophthalmopathy. Invest Ophthalmol Vis Sci 55(8):4935–4943
Hultgren HN (1969) Biomedicine problems of high terrestrial elevations. In: Proceedings of a symposium held at US Army

Research Institute of Environmental Medicine, Massachusetts, USA, 16–17 Oct 1967
Hultgren H (1970) High altitude pulmonary edema. hypoxia, high altitude and the heart. Karger Publishers, Basel, pp 24–31
Hultgren HN, Marticorena EA (1978) High altitude pulmonary edema: epidemiologic observations in Peru. Chest 74(4):372–376
Jain S, Ahmad Y, Bhargava K (2018) Salivary proteome patterns of individuals exposed to high altitude. Arch Oral Biol 96:104–112
Jessen NA et al (2015) The glymphatic system: a beginner's guide. Neurochem Res 40(12):2583–2599
Jessie K, Hashim OH, Rahim ZHA (2008) Protein precipitation method for salivary proteins and rehydration buffer for two-dimensional electrophoresis. Biotechnology 7(4):686–693
Jha KN (2009) Tear break-up time in high altitude areas. Med J Armed Forces India 65(1):2–3
Jha KN (2012) High altitude and the eye. Asia Pac J Ophthalmol (Phila) 1(3):166–169
Johnson TS, Rock PB (1988) Acute mountain sickness. N Engl J Med 319(13):841–845
Juel C et al (2003) Human skeletal muscle and erythrocyte proteins involved in acid-base homeostasis: adaptations to chronic hypoxia. J Physiol 548(2):639–648
Julian CG et al (2013) Exploratory proteomic analysis of hypobaric hypoxia and acute mountain sickness in humans. J Appl Physiol 116(7):937–944
Kaczor-Urbanowicz KE et al (2017) Saliva diagnostics—current views and directions. Exp Biol Med (Maywood) 242(5):459–472
Kakisaka T et al (2007) Plasma proteomics of pancreatic cancer patients by multi-dimensional liquid chromatography and two-dimensional difference gel electrophoresis (2D-DIGE): up-regulation of leucine-rich alpha-2-glycoprotein in pancreatic cancer. J Chromatogr B 852(1–2):257–267
Kami M et al (2001) Use of real-time PCR on blood samples for diagnosis of invasive aspergillosis. Clin Infect Dis 33(9):1504–1512
Kentsis A et al (2010) Discovery and validation of urine markers of acute pediatric appendicitis using high-accuracy mass spectrometry. Ann Emerg Med 55(1):62-70e4
Kharbanda AB et al (2012) Novel serum and urine markers for pediatric appendicitis. Acad Emerg Med 19(1):56–62
Kim HJ et al (2012) Comparison of tear proteins between healthy and early diabetic retinopathy patients. Clin Biochem 45(1–2):60–67
Kumar G et al (2016) H2S regulates hypobaric hypoxia-induced early glio-vascular dysfunction and neuro-pathophysiological effects. EBioMedicine 6:171–189
Lee YH, Wong DT (2009) Saliva: an emerging biofluid for early detection of diseases. Am J Dent 22(4):241–248
Leth-Larsen R, Lund RR, Ditzel HJ (2010) Plasma membrane proteomics and its application in clinical cancer biomarker discovery. Mol Cell Proteomics 9(7):1369–1382
Levett DZ et al (2010) Design and conduct of Caudwell Xtreme Everest: an observational cohort study of variation in human adaptation to progressive environmental hypoxia. BMC Med Res Methodol 10(1):98
Levett DZ et al (2011) The role of nitrogen oxides in human adaptation to hypoxia. Sci Rep 1:109
Li B et al (2014) Tear proteomic analysis of patients with type 2 diabetes and dry eye syndrome by two-dimensional nano-liquid chromatography coupled with tandem mass spectrometry. Invest Ophthalmol Vis Sci 55(1):177–186
Liberti MV, Locasale JW (2016) The Warburg effect: how does it benefit cancer cells? Trends Biochem Sci 41(3):211–218
Lo W-Y et al (2012) miR-27b-regulated TCTP as a novel plasma biomarker for oral cancer: from quantitative proteomics to post-transcriptional study. J Proteom 77:154–166
Lu H, Forbes RA, Verma A (2002) Hypoxia-inducible factor 1 activation by aerobic glycolysis implicates the Warburg effect in carcinogenesis. J Biol Chem 277(26):23111–23115
Lu H et al (2018) Plasma proteomic study of acute mountain sickness susceptible and resistant individuals. Sci Rep 8(1):1265
Luks AM et al (2017) Changes in acute pulmonary vascular responsiveness to hypoxia during a progressive ascent to high altitude (5300 m). Exp Physiol 102(6):711–724
Maggiorini M et al (1990) Prevalence of acute mountain sickness in the Swiss Alps. BMJ 301(6756):853
Mainini V et al (2012) Modulation of urinary peptidome in humans exposed to high altitude hypoxia. Mol BioSyst 8(4):959–966
Maxwell PH (2005) Hypoxia-inducible factor as a physiological regulator. Exp Physiol 90(6):791–797
McLean CJ et al (1989) The effect of high altitude on saliva aldosterone and glucocorticoid concentrations. Eur J Appl Physiol Occup Physiol 58(4):341–347
Mellor A et al (2014) Cardiac biomarkers at high altitude. High Alt Med Biol 15(4):452–458
Mengoli C et al (2009) Use of PCR for diagnosis of invasive aspergillosis: systematic review and meta-analysis. Lancet Infect Dis 9(2):89–96
Michishige F et al (2006) Effect of saliva collection method on the concentration of protein components in saliva. J Med Invest 53(1–2):140–146
Mominzadeh M et al (2014) Stimulated saliva aminotransaminase alteration after experiencing acute hypoxia training. Air Med J 33(4):157–160
Moore LG (2001) Human genetic adaptation to high altitude. High Alt Med Biol 2(2):257–279
Murdoch DR (1995) Altitude illness among tourists flying to 3740 meters elevation in the Nepal Himalayas. J Travel Med 2(4):255–256
Nanjappa V et al (2013) Plasma proteome database as a resource for proteomics research: 2014 update. Nucleic Acids Res 42(D1):D959–D965
Newcombe J et al (1996) PCR of peripheral blood for diagnosis of meningococcal disease. J Clin Microbiol 34(7):1637–1640
Nkuipou-Kenfack E et al (2015) Identification of ageing-associated naturally occurring peptides in human urine. Oncotarget 6(33):34106–34117
Omenn GS (2006) Strategies for plasma proteomic profiling of cancers. Proteomics 6(20):5662–5673
Padhy G et al (2016a) Plasma kallikrein-bradykinin pathway promotes circulatory nitric oxide metabolite availability during hypoxia. Nitric Oxide 55–56:36–44
Padhy G et al (2016b) Plasma kallikrein-bradykinin pathway promotes circulatory nitric oxide metabolite availability during hypoxia. Nitric Oxide 55:36–44
Pan S et al (2011) Protein alterations associated with pancreatic cancer and chronic pancreatitis found in human plasma using global quantitative proteomics profiling. J Proteome Res 10(5):2359–2376
Paul S, Bhargava K, Ahmad Y (2017) The meta-analytical paradigm in an in silico hybrid: pathways and networks perturbed during exposure to varying degrees of hypobaric hypoxia. Proteomics Clin Appl 11(7–8):1600160
Peluso G et al (2007) Proteomic study of salivary peptides and proteins in patients with Sjogren's syndrome before and after pilocarpine treatment. Arthritis Rheum 56(7):2216–2222
Pfaffe T et al (2011) Diagnostic potential of saliva: current state and future applications. Clin Chem 57(5):675–687
Pieragostino D et al (2012) Differential protein expression in tears of patients with primary open angle and pseudoexfoliative glaucoma. Mol BioSyst 8(4):1017–1028

Pilardeau P et al (1990) Saliva flow and composition in humans exposed to acute altitude hypoxia. Eur J Appl Physiol Occup Physiol 59(6):450–453
Pleitez M, von Lilienfeld-Toal H, Mantele W (2012) Infrared spectroscopic analysis of human interstitial fluid in vitro and in vivo using FT-IR spectroscopy and pulsed quantum cascade lasers (QCL): establishing a new approach to non invasive glucose measurement. Spectrochim Acta A Mol Biomol Spectrosc 85(1):61–65
Plog BA et al (2015) Biomarkers of traumatic injury are transported from brain to blood via the glymphatic system. J Neurosci 35(2):518–526
Poff AM et al (2013) The ketogenic diet and hyperbaric oxygen therapy prolong survival in mice with systemic metastatic cancer. PLoS One 8(6):e65522
Raimondo F et al (2014) The urinary proteome and peptidome of renal cell carcinoma patients: a comparison of different techniques. Expert Rev Proteomics 11(4):503–514
Rajput C et al (2006) Endothelin-1 gene variants and levels associate with adaptation to hypobaric hypoxia in high-altitude natives. Biochem Biophys Res Commun 341(4):1218–1224
Rennie D et al (1972) Urine and plasma proteins in men at 5,400 m. J Appl Physiol 32(3):369–373
Reuter S et al (2010) Oxidative stress, inflammation, and cancer: how are they linked? Free Radic Biol Med 49(11):1603–1616
Roach RC, Hackett PH (2001) Frontiers of hypoxia research: acute mountain sickness. J Exp Biol 204(18):3161–3170
Roach R et al (2000) Exercise exacerbates acute mountain sickness at simulated high altitude. J Appl Physiol 88(2):581–585
Sartori C et al (1999) Exaggerated endothelin release in high-altitude pulmonary edema. Circulation 99(20):2665–2668
Schafer CA et al (2014) Saliva diagnostics: utilizing oral fluids to determine health status. Monogr Oral Sci 24:88–98
Scherrer U et al (2010) New insights in the pathogenesis of high-altitude pulmonary edema. Prog Cardiovasc Dis 52(6):485–492
Schiffer E et al (2012) Urinary proteome analysis for prostate cancer diagnosis: cost-effective application in routine clinical practice in Germany. Int J Urol 19(2):118–125
Seen S, Tong L (2018) Dry eye disease and oxidative stress. Acta Ophthalmol 96(4):e412–e420
Semenza GL (2004) Hydroxylation of HIF-1: oxygen sensing at the molecular level. Physiology 19(4):176–182
Siervo M et al (2014) Effects of prolonged exposure to hypobaric hypoxia on oxidative stress, inflammation and gluco-insular regulation: the not-so-sweet price for good regulation. PLoS One 9(4):e94915
Sikri G, Bhattachar S (2017) Comment on "soluble urokinase-type plasminogen activator receptor plasma concentration may predict susceptibility to high altitude pulmonary edema". Mediat Inflamm 2017:8546027. https://doi.org/10.1155/2017/8546027
Singh I et al (1969) Acute mountain sickness. N Engl J Med 280(4):175–184
Stevens S (2011) Schirmer's test. Community Eye Health 24(76):45
Streckfus CF, Bigler L (2016) A catalogue of altered salivary proteins secondary to invasive ductal carcinoma: a novel in vivo paradigm to assess breast cancer progression. Sci Rep 6:30800
Streckfus CF et al (2008) Breast cancer related proteins are present in saliva and are modulated secondary to ductal carcinoma in situ of the breast. Cancer Invest 26(2):159–167
Sun X et al (2016) Comparative proteomic profiling identifies potential prognostic factors for human clear cell renal cell carcinoma. Oncol Rep 36(6):3131–3138
Thomas CE et al (2010) Urine collection and processing for protein biomarker discovery and quantification. Cancer Epidemiol Biomark Prev 19(4):953–959
Torok Z et al (2015) Combined methods for diabetic retinopathy screening, using retina photographs and tear fluid proteomics biomarkers. J Diabetes Res 2015:623619
Tyagi T et al (2014) Altered expression of platelet proteins and calpain activity mediate hypoxia-induced prothrombotic phenotype. Blood 123(8):1250–1260
van Patot MCT et al (2005) Greater free plasma VEGF and lower soluble VEGF receptor-1 in acute mountain sickness. J Appl Physiol 98:1626–1629
Vaupel P, Mayer A (2007) Hypoxia in cancer: significance and impact on clinical outcome. Cancer Metastasis Rev 26(2):225–239
Wang LT et al (2007) The use of white blood cell count and left shift in the diagnosis of appendicitis in children. Pediatr Emerg Care 23(2):69–76
Wang J et al (2009) MicroRNAs in plasma of pancreatic ductal adenocarcinoma patients as novel blood-based biomarkers of disease. Cancer Prevent Res 2(9):807–813
Wayner D et al (1985) Quantitative measurement of the total, peroxyl radical-trapping antioxidant capability of human blood plasma by controlled peroxidation: the important contribution made by plasma proteins. FEBS Lett 187(1):33–37
Wellinghausen N et al (2009) Diagnosis of bacteremia in whole-blood samples by use of a commercial universal 16S rRNA gene-based PCR and sequence analysis. J Clin Microbiol 47(9):2759–2765
Willmann G et al (2014) Exposure to high altitude alters tear film osmolarity and breakup time. High Alt Med Biol 15(2):203–207
Wilson WR, Hay MP (2011) Targeting hypoxia in cancer therapy. Nat Rev Cancer 11(6):393
Woods DR et al (2012) The cortisol response to hypobaric hypoxia at rest and post-exercise. Horm Metab Res 44(4):302–305
Wright CF, Burton H (2008) The use of cell-free fetal nucleic acids in maternal blood for non-invasive prenatal diagnosis. Hum Reprod Update 15(1):139–151
Xiao H et al (2012) Proteomic analysis of human saliva from lung cancer patients using two-dimensional difference gel electrophoresis and mass spectrometry. Mol Cell Proteomics 11(2):M111 012112
Xing G et al (2008) Adaptation and mal-adaptation to ambient hypoxia; Andean, Ethiopian and Himalayan patterns. PLoS One 3(6):e2342
Yaari A et al (2006) Detection of HCV salivary antibodies by a simple and rapid test. J Virol Methods 133(1):1–5
Yoon KC et al (2010) Expression of CXCL9, -10, -11, and CXCR182 in the tear film and ocular surface of patients with dry eye syndrome. Invest Ophthalmol Vis Sci 51(2):643–650
Yoshizawa JM et al (2013) Salivary biomarkers: toward future clinical and diagnostic utilities. Clin Microbiol Rev 26(4):781–791
Young BL et al (2014) The identification of tuberculosis biomarkers in human urine samples. Eur Respir J 43(6):1719–1729
Zhang Q, Faca V, Hanash S (2010) Mining the plasma proteome for disease applications across seven logs of protein abundance. J Proteome Res 10(1):46–50
Zhang Y et al (2013) Proteomic identification of human serum biomarkers associated with high altitude pulmonary edema. Chin J Appl Physiol 29(6):501–507
Zhang ZY et al (2015a) Urinary proteome and systolic blood pressure as predictors of 5-year cardiovascular and cardiac outcomes in a general population. Hypertension 66(1):52–60
Zhang M, Fu G, Lei T (2015b) Two urinary peptides associated closely with type 2 diabetes mellitus. PLoS One 10(4):e0122950
Zhou L, Beuerman RW (2012) Tear analysis in ocular surface diseases. Prog Retin Eye Res 31(6):527–550
Zhou L, Beuerman RW (2017) The power of tears: how tear proteomics research could revolutionize the clinic. Expert Rev Proteomics 14(3):189–191

Zhou H et al (2006) Collection, storage, preservation, and normalization of human urinary exosomes for biomarker discovery. Kidney Int 69(8):1471–1476

Zhou L et al (2009) Identification of tear fluid biomarkers in dry eye syndrome using iTRAQ quantitative proteomics. J Proteome Res 8(11):4889–4905

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