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Polyamine Biomarkers as Indicators of Human Disease

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ABSTRACT

The significant increase of periodontitis, chronic kidney disease (CKD), Alzheimer's disease and cancer can be attributed to an ageing population. Each disease produces a range of biomarkers that can be indicative of disease onset and progression. Biomarkers are defined as cellular (intra/extracellular components and whole cells), biochemical (metabolites, ions and toxins) or molecular (nucleic acids, proteins and lipids) alterations which are measurable in biological media such as human tissues, cells or fluids. An interesting group of biomarkers that merit further investigation are the polyamines. The polyamines are a group of molecules consisting of cadaverine, putrescine, spermine and spermidine and these have been implicated in the development of a range of systemic disease, in part due to their production in periodontitis. Cadaverine and putrescine within the periodontal environment have demonstrated cell signalling interfering abilities, by way of leukocyte migration disruption. The polyamines spermine and spermidine in tumour cells have been shown to inhibit cellular apoptosis, effectively prolonging tumorigenesis and continuation of cancer within the host. Polyamine degradation products such as acrolein have been shown to exacerbate renal damage in CKD patients. Thus, the use of such molecules has merit to be utilised in the early indication of such diseases in patients.

Keywords: Biomarkers; Periodontitis; Polyamines; Chronic Kidney Disease; Alzheimer's Disease; Cancer.

1.1 Introduction

Periodontitis, chronic kidney disease, Alzheimer's disease, and cancer represent some of the most prevalent diseases within human populations. There is an urgent need to detect such diseases in their early stages, before they are able to progress and cause harm. The utilisation of disease specific biomarkers may provide valuable diagnostic information which can aid clinical decision making thus, increasing the potential of early interventions. Polyamines demonstrate a potential group of biomarkers which have been suggested to provide clinical evidence of active disease. Furthermore, oral diseases, namely periodontitis, have also been suggested to demonstrate significant correlations towards the incidence of systemic diseases (diseases which may be influenced due to transient bacteraemia and the distal deposition of oral microbial metabolites such as the polyamines) resulting in the metastatic spread of infection which induces systemic inflammatory reactions. Thus, the utilisation of polyamine based biomarkers in oral fluids may be a potential application that requires further exploration.

1.1.1 The oral cavity and its microenvironment

The oral cavity is of a unique design, whereby the juxtaposition of hard and soft tissues are continually exposed and challenged by numerous external material pressures (Taylor and Preshaw, 2016). It is one of the most complex regions on the human body and plays a vital role for providing entry, transit and exit for the digestive and respiratory systems, and it aids in the mastication and chemical pre-processing of foods (Yven et al., 2006). Within the oral cavity, there are hard tissues such as the teeth which are lined by the gingiva or the mucosa, and also soft tissue structures such as the cheeks, soft and hard palate and the tongue along with the periodontium, which is a collection of specialised connective tissues. The periodontium is a collective term which describes the tooth supporting and investing tissues such as the gingiva, root cementum, periodontal ligament and alveolar bone (Cho and Garant, 2000). This

significant number and variety of structures within the oral cavity allow for the colonisation of a substantial number of microorganisms on the oral surfaces.

In the oral cavity, there are two key biofluids present, these being saliva and gingival crevicular fluid (GCF). Saliva is a complex fluid which is secreted from three major glands in the mouth (90% total saliva production), along with a large quantity of minor glands (10% of total saliva production) (de Almeida et al., 2008). Secretion of the saliva from the salivary glands is a response mediated by the autonomic nervous system (Sarapur and Shilpashree, 2012).

1.1.2 Oral biofluids

The role of saliva is to protect the teeth, oral and peri-oral tissues, and to facilitate eating and speech (Dodds et al., 2015). Saliva is composed of a number of different electrolytes, including sodium, bicarbonates and phosphates, as well as immune complexes such as immunoglobulins, proteins and mucins (Humphrey and Williamson, 2001). The production of saliva is increased significantly upon the acknowledgment of food in the mouth through both mechanical and chemical stimuli (Neyraud et al., 2003). Both visual and olfactory stimuli have also been shown to increase saliva production (Keesman et al., 2016). In the oral cavity, saliva also acts as a lubricant, aiding in the initial digestion of foods through the formation of bolus for swallowing and initiation of the digestion of food components (Pedersen et al., 2002). Furthermore, due to the continual bathing of saliva in the mouth, this has shown to have a profound influence on the oral ecology and resulting microenvironment.

The GCF is an oral inflammatory exudate, which is derived from the periodontal tissues, and is found in the sulcus between the tooth and the gingiva (Lamster, 1997; Subbarao et al., 2019). Its role is to facilitate the antimicrobial defence of the periodontium and to maintain the structure of the junctional epithelium (Subbarao et al., 2019). Under normal

conditions, GCF is sourced in small volumes of 0.43 – 1.56 $\mu\text{L/h}$ (Khurshid et al., 2017). However, the amount of GCF produced significantly increases, up to 44 $\mu\text{L/h}$ in response to stimuli from the immune system and during periodontal disease. The constituents of GCF originate from the blood, surrounding cells and the various tissues of the periodontium (Lamster and Ahlo, 2007). Although the role of GCF is to prevent microbial mediated damage to the oral cavity, an increase in GCF has been shown to increase localised nutrients and provide a suitable physical environment for periodontal microorganisms, thus developing a positive feedback loop (Hickey et al., 2020).

1.1.3 Oral microbiome

The oral microbiome is defined as the entire genome of microorganisms that reside within the oral cavity and it provides the second largest microbial community in humans (Deo and Deshmukh, 2019; Gao et al., 2018). It consists of a core microbiome and a variable microbiome. The core microbiome is common amongst all individuals, whereas the variable microbiome changes are dependent on the lifestyle of the individual and physiological pressures (Kilian et al., 2016). There are two types of surfaces which bacteria in the mouth are able to effectively colonise, the hard tissues such as the teeth, and soft tissues of the oral mucosa (Deo and Deshmukh, 2019). Once initial colonisation of a surface occurs, bacterial proliferation develops into a bacterial biofilm.

Comprised of over 700 diverse species of microorganisms, the oral cavity is a host for bacteria, fungi, mycoplasma and protozoa (Kuramitsu et al., 2007). The oral microbiota is imperative in the normal development of the host, effectively contributing to host defences, synthesis of important vitamins such as vitamin B and K and aiding in digestion. The oral microbiota is also involved in the prevention of exogenous pathogenic microorganisms, thus the relationship between host and oral microbiota is not one which is singularly passive (Patil

et al., 2013; Marsh, 2009). In terms of mutual and functional integration, the relationship and multifaceted balance between host and oral microenvironment determines the health status of the oral cavity (Cornejo Ulloa et al., 2019).

1.2.1 Periodontitis

Periodontitis is an orally, microbial driven inflammatory disease of the periodontium (Hajishengallis, 2015). The disease typically manifests as reoccurring inflammation of the gingiva, gingiva bleeding and the formation of periodontal pockets (Shatzle et al., 2004). It is estimated that between 20% – 50% of the global adult population are afflicted with periodontitis, making it the sixth most prevalent disease worldwide (Nazir, 2017). Periodontitis results in eventual loss of the periodontal ligament and subsequent destruction of alveolar bone by compromising the integrity of supporting tooth structures (Fig 1) (de Pablo et al., 2009; Al Moharib et al., 2014). From initiation, the disease progresses with the detachment of collagen fibres from the root cementum, apical migration of the junctional epithelium, deepened pocket formation and finally, resorption of the alveolar bone (Tsuchida et al., 2017). Periodontitis will ultimately progress to bone destruction if untreated, leading to increased tooth mobility with subsequent tooth loss (Hienz et al., 2015).

Figure 1. The decline of the healthy tooth and periodontium with the onset of gingivitis, leading to periodontitis.

130

131 *1.2.2 Periodontitis and oral microorganisms*

132 Dental bacterial biofilms (also known as plaques) are the main aetiological agents
 133 which causes gingival inflammation to progress to periodontitis (Hajishengallis, 2015). These
 134 dental plaques, associated with changes in the bacterial species, dysregulate the normal oral
 135 microbiota and as a result cause the inflammatory response of which periodontitis is indicative
 136 (Wahid et al., 2013). This increased microbial colonisation results in an increase in GCF flow,
 137 which anaerobic bacteria have been shown to favour. Anaerobic microorganisms which include
 138 *Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola* have been shown
 139 to be the most prevalent anaerobic bacteria associated with human periodontitis and they are
 140 frequently isolated in the majority of cases. These periodontopathogens alongside other “key
 141 accessory” microorganisms have been categorised into complexes based on their chronological
 142 colonisation of the gingival crevice and virulence in the subgingival plaques (Table 1).

143

144 **Table 1.** Subgingival bacterial classification in Socransky complexes (Socransky et al., 1998).

Bacterial Species	Complex
<i>Streptococcus gordonii</i> <i>Streptococcus intermedius</i> <i>Streptococcus mitis</i> <i>Streptococcus sanguinis</i>	Yellow
<i>Campylobacter rectus</i> <i>Fusobacterium nucleatum</i> <i>Peptostreptococcus micros</i> <i>Prevotella intermedia</i>	Orange
<i>Actinobacillus actinomycetemcomitans</i> <i>Tannerella forsythia</i> <i>Porphyromonas gingivalis</i> <i>Treponema denticola</i>	Red

145

146

147 The red complex bacteria encompass what is known to be the most pathogenic bacteria
148 in human periodontal disease (Suzuki et al., 2013). These Gram negative bacteria become more
149 prevalent during the later stages of dental biofilm development (Kesavalu et al., 2007). Studies
150 have shown that there is an upwards of 96% infection rates of *P. gingivalis*, of which, 75% are
151 in active periodontal sites, and 59.7% of *P. gingivalis* colonisation is found in inactive regions
152 (Hernández et al., 2011; López, 2000). The colonisation of these areas by such microorganisms
153 enables the initiation of inflammation to develop in the surrounding tissues, which can result
154 in the loss of connective tissues and alveolar bone. This facilitates the conversion of the
155 junctional epithelium to pocket epithelium (Bosshardt, 2018).

156

157 *1.2.3 Dysbiosis in periodontal disease*

158 Historically it has been thought that the red complex microorganisms were entirely
159 responsible for the dysbiosis observed in the normal oral microflora through dysregulation of
160 cell signalling pathways (Darveau et al., 2002). However, this understanding has since evolved,
161 and it has now been suggested that periodontitis is a much more complex disease and is not
162 solely regulated by one group of microorganisms. As a result, the descriptor of periodontitis
163 infection is now based on the polymicrobial synergy and dysbiosis model (PSD) (Wang, 2015;
164 Hajishengallis and Lamont, 2012). This model specifically refers to the communication
165 between metabolically compatible microorganisms within an environment, which acquire
166 functional specialisation through synergistic activities (Shaikh et al., 2018). The gingival
167 crevice is colonised by an assembly of compatible microorganisms, grouped into heterotypic
168 communities, to which the red complex bacteria and key accessory microorganisms interact.
169 Such interactions increase community virulence and result in dysbiosis and tissue homeostasis
170 disruption, causing the destruction of periodontal tissues (Hajishengallis and Lamont, 2012).

However, the bacteria at a specific region of the oral cavity change in relation to the tissues and structures to which they are bound and also to local differences in environmental pressures. An example of this occurs in fissures, on proximal tooth surfaces and in the gingival crevice, where two distinct variations of bacterial accumulations can be observed (Lamont and Hajishengallis, 2015).

The infection of the periodontal tissues presents a potential portal to enable periodontal microorganisms, bacterial metabolites and further biomarkers such as antigens into the body. Such bacteria and molecules may promote disease elsewhere in the body *via* dissemination of the circulatory system (Hickey et al., 2020). Such is evident in cases of Alzheimer's disease, and chronic kidney infections (Laugisch et al., 2018; Wahid et al., 2013).

2.1 The importance of biomarkers in human disease

A biomarker is defined as a biochemical, cellular or molecular alteration which is measurable in any biological media such as the human tissues, cells or fluids (Mayeux, 2004). Biomarkers are able to represent an objective indication of the medical state on an individual, and can be observed outside of the body, and measured with precision whilst maintaining a high degree of replicability (Strimbu and Tavel, 2010; Choong and Tsafnat, 2012). In the late 1990's, the National Institute of Biomarker Definitions working group described a biomarker as 'a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention' (Strimbu and Tavel, 2010). These biomarkers were henceforth divided into two distinct categories: exposure biomarkers, which enable the assessment of potential health risks, and disease biomarkers, which are currently in use for screening, diagnostics and monitoring existing diseases (Hwang et al., 2018; Strimbu and Tavel, 2010; Lowry, 1995). In order for a

biomarker to demonstrate a clear predictive value in which a disease or biochemical process can be effectively measured, definitive values for the biomarker need to be established.

2.1.1 Existing uses of biomarkers

In modern medicine, biomarkers are frequently utilised for screening of diseases such as Alzheimer's disease, cancer and periodontal diseases (Table 2) (Goossens et al., 2015; Sharma and Singh, 2016). Within such diseases, the uses of biomarkers may allow for confirmation of a diagnosis, initial detection of the suspected disease, or for monitoring the outcomes of therapeutic interventions (Selleck et al., 2017). Biomarker research is now at the point where there is significant research input in the field of new biomarker discovery, and subsequently, biomarkers are being constantly developed and refined. This has resulted in significant breakthroughs in the fields of drug discovery, clinical trials, epidemiology and personalised medicine (Collinson, 2013; White and Xie, 2013).

Table 2. Existing biomarkers used for the detection of current human diseases (Stathopoulou et al., 2015; Lopez-Giacoman and Madero, 2015; Sauter, 2017; Sharma and Singh, 2016).

Alzhiemers disease	Cancer	Kidney disease	Periodntal disease
Tau protein	ctDNA (oral cancer)	Serum creatinine	Lypopolysaccarides
Phosporylated tau protein	Cytokeratin (in 90% of breast cancers)	Urinary albumin	Matrix metalloproteases
Amyloid B plaques	Ki-67 (cellular proliferation)	Cystatin C	Serum IgG

For biomarkers in human disease to be effectively measured, they must be detected within their respective biofluids. Current biofluids such as blood plasma, serum, urine and cerebrospinal fluid are routinely used as a source of detecting biomarkers indicative of disease (Xu and Veenstra, 2008; Kulic and Unschuld, 2016). The ability to effectively replicate measurements of biomarkers to a high degree of accuracy and sensitivity has been at the forefront of medical and epidemiological research (Thyagarajan et al., 2016; Tarnanas et al., 2015). Within humans, the degree of biomarker variability between people results in a range of acceptable ‘normal’ values. In such fluids, biomarkers are used as indicators of a clinical manifestation, disease stage, or to determine an alternate manifestation of the disease in question (Mayeux, 2004). It is extremely important that the surrounding biofluid does not interfere with the signal produced by the selected biomarker. Previous studies have shown

that although biomarkers may potentially have the capability to demonstrate levels which may be indicative of disease association, they may demonstrate low levels of repeatability (Thyagarajan et al., 2016). Thus the reproducibility of biomarker measurements when used for disease detection is still of concern (Yeh et al., 2017). However, it is evident that biomarkers potentially have significant advantages to be used in disease detection over traditional diagnostic methods although consideration needs to be given to the natural variability between human participants.

2.1.2 Infection biomarkers of bacterial driven disease

The use of biomarkers where typical diagnosis methods are insufficient, expensive and/or time consuming, may result in the most successful application for the use of biomarkers (Lubell and Althaus, 2017). Since biomarkers are able to provide a clinician with valuable diagnostic and prognostic information regarding the current health status of an individual, their applicability in various bacterially driven diseases is of major importance (Tang et al., 2017; Gomez et al., 2019). For example, tuberculosis (TB), is a communicable infectious disease that is known for having a long incubation time (2-8 weeks), resulting in severely delayed confirmation diagnosis. This is due to the detection methods relying on the bacteria being cultured to provide a positive confirmation. However, new biomarkers in the form of lipoarabinomannan, a virulence factor and glycolipid of the cell wall of the causative agent of TB (*Mycobacterium tuberculosis*) has shown good specificity to determine the presence of TB in the blood sputum and urine, without the use of bacterial cultures (Goletti et al., 2016; Correia-Neves et al., 2019; Wallis et al., 2010). This example demonstrates the effectiveness of the potential of new biomarkers as rapid detection alternatives. The use of such systems have the possibility to reduce diagnosis times whilst maintaining comparable levels of diagnostic accuracy to that of traditional techniques.

258

259 ***3.1 Periodontal disease and its associated biomarkers***

260 Periodontal disease is a prevalent disease afflicting a significant proportion of the
261 human population, thus new rapid methods of detection and quantification that are non-
262 invasive would be advantageous for use in patients rather than the use of traditional invasive
263 interventions. Traditional methods of periodontal disease assessments make use of techniques
264 such as bleeding on probing, pocket depth analysis, clinical attachment levels (CAL), plaque
265 indexes, and analysis of radiographs of current alveolar bone levels (Fig 2) (Preshaw, 2015).
266 These traditional measures are useful in the assessment of patient disease history but provide
267 little to no information about the patient's current health, the active state of the disease, or risk
268 of potential future periodontal breakdown (Srivastava et al., 2017). Furthermore, as the gingival
269 pocket becomes more pronounced with disease progression, probing the area is painful for the
270 patient. The use of a rapid biomarker detection system that relies on simply touching an area
271 of the gum would provide a more rapid and less painful procedure for the patient.

272 The current aim for biomarkers in the field of periodontal research is to develop rapid,
273 high impact diagnostics, which enhance clinical decision making. This should result in
274 affordable, economically viable healthcare and an increase in favourable patient outcomes
275 (Urdea et al., 2011). For such biomarker-based tests/measurements to become widely utilised,
276 they must be at least of equal calibre to existing clinical diagnostics but show improvements in
277 saving time, cost and also be easy to use for both the user and recipient.

278

Figure 2. Clinical means of periodontal disease assessment utilising traditional methods of bleeding on probing, probing depths and clinical attachment levels (Taba et al., 2005).

3.1.1 Saliva and GCF in periodontal disease

It has been suggested that the saliva and GCF can be used to determine the periodontal health status of an individual since the oral fluids contain specific disease biomarkers indicative of periodontitis (Patil and Patil, 2011). Saliva which is readily available and collected without invasive interventions provides an ideal source of periodontal biomarkers. Molecules are able to be transported either into or out of the saliva and GCF through cells *via* passive diffusion, active transport, or by extracellular ultra-filtration (Srivastava et al., 2017). When individuals present with chronic inflammation, which accompanies periodontitis, the saliva becomes host to a myriad of biomarkers. In recent years, the use of GCF has also gained wide interest as a source of biomarkers for periodontal disease since it shows clear changes during different stages of periodontal disease progression (Majeed et al., 2016; Ghallab, 2018; Barros et al., 2016). Collection of GCF is a non-invasive procedure and it is simple to collect from individuals with underlying comorbidities, since it is an inflammatory exudate which increases

in volume in response to a periodontal infection. Thus, both saliva and GCF appear to be attractive options for use as diagnostic fluids.

3.1.2 Biomolecules for periodontal disease detection

Periodontal disease can be attributed to the presence of certain bacteria species and as such, there has been numerous studies suggesting that the presence of antibodies directed towards the detection of these bacteria may implement new strategies for targeted biomarkers. In both the GCF and saliva of periodontitis patients, the presence of IgA and IgG antibodies have been sourced towards four periodontal disease specific bacteria, *P. gingivalis*, *A. actinomycetemcomitans*, *Prevotella intermedia* and *Fusobacterium nucleatum*. Similar results between each bacterium were found, whereby a skewed pattern of significantly elevated levels of bacteria specific IgG and IgA antibodies were detected when compared to healthy controls (Plombas et al., 2002). Moreover, further studies compared 50 healthy individuals and 50 periodontitis afflicted patients. Significantly elevated levels of IgA and IgG antibodies respective to four keystone periodontal pathogens (*P. gingivalis*, *T. denticola*, *T. forsythia* and *A. actinomycetemcomitans*) were identified and demonstrated a periodontitis stage dependant increase in antibody concentration (Gadekar et al., 2018; Dye et al., 2009). Thus, IgA and IgG antibodies specific to keystone periodontitis pathogens in the GCF and to a lesser extent the saliva, are potentially able to indicate the potential ‘at risk’ sites for periodontitis due to their increased concentration (Takahashi et al., 1997).

The majority of damaging interactions which occur in periodontal disease take place at the crevicular and junctional epithelium (Fujita et al., 2018). Biomolecules, such as enzymes, endotoxins, nucleic acids, proteins, carbohydrates, degradation products and immunoglobulins, which result from periodontal bacteria residing in the GCF, have been shown to induce significant host tissue damage (Cekici et al., 2014; Barnes et al., 2014). Recent evidence would

suggest that the host inflammatory response to periodontal bacteria may also aggravate periodontal disease and demonstrate commonly identified disease specific pathologies (Nędzi-Góra et al., 2017). It has been demonstrated that under activation due to numerous chemical signalling molecules, polymorphonuclear leukocytes produce increasing levels of reactive oxygen species and proteolytic enzymes (Mariggiò et al., 2004). The hyperactivity of this response significantly contributes to the host tissue destruction during periods of active periodontal disease (Nair et al., 2014). During this host pathogen interaction many free amino acids and metabolites are significantly elevated (Barnes et al., 2009). Such elevation of potential biomarkers due to the mechanisms of infection mean that the whole saliva and GCF can provide a large, easily accessible pool of biomolecules for which periodontal disease detection and staging can potentially be evaluated. Further study is warranted in order to optimise the repeatability of measurements taken using such biomarkers and to determine how such markers are directly correlated with disease extent and progression.

4.1 Polyamines and periodontal disease

The colonisation of bacteria into gingival tissues is limited through salivary flow and muscular movements (Pedersen and Belstrøm, 2019). During a periodontal infection, the presence of GCF is increased and provides a more favourable environment for the formation of dental biofilms (Marsh, 2003). Over the course of infection, the composition of the sulcular dental biofilm composition shifts, from a mostly Gram positive and saccharolytic microflora, to a Gram negative, proteolytic microbiota (Berezow and Darveau, 2011). The Gram negative “keystone” bacteria are able to exacerbate the inflammatory events occurring in the periodontium through the release of biomolecules. One potential class of molecules, the polyamines, may be utilised as potential biomarkers to determine the incidence and severity of a periodontal infection.

4.1.1 Polyamine biosynthesis

The polyamines, cadaverine, putrescine, spermine and spermidine, are a group of organic polycationic molecules which are required for growth and differentiation of almost every eukaryotic and prokaryotic cell (Fig 3) (Takahashi and Kakehi, 2010; Shah and Swiatlo, 2008).

Figure 3. Biosynthetic pathways demonstrating the degradation of the biogenic amine, putrescine into its respective secondary and tertiary polyamines, spermidine and spermine.

These aliphatic polycationic molecules are ubiquitous in all tissues and cells in both plants and animals. Their roles within humans, span a broad range of functions, and these encompasses influencing cell apoptosis, division, differentiation, proliferation, DNA and protein synthesis, gene expression signal transduction and homeostasis (Fig 4) (Kusano et al., 2008; Pegg, 2016; Handa et al., 2018). Based on cellular distribution alone, putrescine and

spermine are the most prominent within human biosystems, aiding in cellular growth and division in both eukaryotic and prokaryotic cells (Handa et al., 2018).

Figure 4. Polyamines express various functions upon entering the cell. The nucleus, mitochondria and cytoplasm are influenced differently, although, polyamines are a necessity for each cell component. Polyamines are involved in the regulation cell proliferation and termination, and also gene expression and translation. There is now increasing evidence to suggest that polyamines play a role in cellular reprogramming and autophagy.

4.1.2 Polyamine in periodontal disease

Cadaverine and putrescine have been identified as upregulated biomolecules in periodontal disease and their role in the pathogenesis of periodontal disease has been suggested in a number of previous studies (Lamster et al., 1987; Mariggio et al., 2004; Lohinai et al., 2012). Despite their abundance in cells, polyamine levels are tightly regulated. However, there are some differences in the concentrations of certain polyamines such as cadaverine when measured in the saliva of individuals. Tábi et al., (2008) measured average cadaverine levels that increased to $11.8 \pm 8.30 \mu\text{M}$, from $7.9 \pm 6.48 \mu\text{M}$, after oral hygiene was restricted and they concluded that such an increase in cadaverine concentration could potentially contribute to an increase in the development of periodontal diseases. Fine and Mandel, (1986) described increases of up to 10 fold of cadaverine in patients whose plaque index score was measured from 1 to 2, leading to suggestions that cadaverine was the best indicator to measure the metabolic activity of plaque which is associated with the onset of periodontal disease. The upregulation of cadaverine has been observed by Lohinai et al (2012), through the measurements of human dental biofilms. The concentrations of cadaverine, lysine and lysine decarboxylase in dental biofilms after one week of oral hygiene restriction were measured as a determination of damage to the gingival sulci. Cadaverine in this instance was produced as a result of lysine decarboxylation and the cells attached to the dental tissues become lysine deprived. This results in the release of pro-inflammatory cytokines, which act on the sub-epithelial blood vessels to become permeabilised, or experience autophagy. This enables the dental biofilm constituents to access the gingival stroma to release cytokines and initiate GCF exudation.

The use of polyamines as potential biomarkers has been suggested in a number of studies (Nakajima et al., 2018; Park and Igarashi, 2013; Sakanaka et al., 2017). Polyamines such as putrescine and cadaverine, are frequently detected at increased levels in the GCF of

periodontitis afflicted individuals, which was made evident by Walters et al, (1987) whereby, 19 patients with mild to moderate periodontal disease measured a mean concentration of 921 μ M and 615 μ M for putrescine and cadaverine in the GCF respectively. The use of cadaverine specifically as a salivary biomarker for periodontal inflammatory status may be a possibility, since results have been shown to coincide with findings from previous metabolomics analyses of saliva from patients with predetermined periodontal disease (Barnes et al., 2014). The presence of cadaverine in human biofluid at greater than physiological concentrations is something typically introduced *via* an external source. Sakanaka et al (2017), postulated that cadaverine is actively metabolised by periodontal microorganisms due to significantly increased concentrations during periodontitis, thus its presence in saliva can be correlated to the number of bacteria colonising a specific region of the oral cavity. Furthermore, during metabolomics studies conducted using saliva from a periodontal patient, it was shown that a greater abundance of cadaverine was found in patients with an increased periodontally inflamed surface area (PISA), and the increase was attributed to putrefaction and breakdown of lysine via the oral bacteria.

Work by Lohinai et al., (2012), suggests that the dental biofilm site represents a significant location of cadaverine production. Since cadaverine is not typically present in healthy human blood, it has been suggested that the cadaverine from the dental biofilm diffuses from the site into the saliva, whereby it also penetrates tongue biofilms and the surrounding gingival sulci. In a case study by Lamster et al (1987), polyamine analysis of the GCF was conducted before and after two weeks of mucoperiosteal flap surgery on patients whom exhibited periodontitis. These areas demonstrated high values of putrescine pre-operatively (1020 pmol/mL). Similarly, putrescine values were observed at one week and two weeks post operatively (124 pmol/mL and 880 pmol/mL respectively). The polyamine content in the supragingival plaque of periodontitis afflicted individuals showed putrescine (1.49 nmol/mg)

was in the highest abundance, followed by spermine (0.03 nmol/mg) and spermidine (0.90 nmol/mg). This reduction in the concentration of spermine and spermidine was to be expected, since the direct degradation of putrescine leads to the formation of spermidine and spermine respectively (Pegg, 2016). The measure of putrescine in GCF provided better differentiation between relative periodontal health and diseased states since it was observed to be present at significantly higher levels in deep periodontal pocket sites in comparison to healthy controls (Ozeki et al., 2016; Lamster et al., 1987). Overall, it was suggested that the ability to quantify putrescine, spermine and spermidine within active periodontal sites was possible.

4.1.3 Polyamines in bacteria

Putrescine, cadaverine, spermidine and spermine are the predominant polyamines which are found in bacteria (Guerra et al., 2018). The role of bacterially derived polyamines has been shown to be associated with cell metabolism, cell-to-cell communication and bacterial cell differentiation, whilst also significantly contributing towards bacterial signalling, motility, and cell division (Kurihara et al., 2005; Igarashi and Kashiwagi, 2000; Miller-Fleming et al., 2015). Putrescine has also been determined to constitute to the outer membrane walls of some Gram negative bacteria such as *Salmonella enterica* and *Proteus mirabilis*, and cadaverine has been associated with the peptidoglycan of *Veillonella* spp. suggesting the importance of polyamines of maintaining the outer surface structures of bacteria (Shah and Swiatlo, 2008; Vinogradov and Perry, 2000; Kamio, 1987). Their synthesis relies on the presence of functional precursor molecules, similar to humans and these are detected at millimolar concentrations in bacteria (Tofalo et al., 2019). The intracellular concentrations of spermidine are determined to be the highest in bacteria at 1 – 3 mM, whilst putrescine demonstrate the lowest levels at 0.1 – 0.2 mM (Shah and Swiatlo, 2008).

Recent studies have investigated the impact of polyamines in bacteria (Goforth et al., 2013; Nakamya et al., 2018). Polyamines are suggested to play a vital role in aiding the pathogenesis of bacterial species. Cadaverine has been implicated in enhancing oral bacterial proliferation through the inhibition of leukocytes thus decreasing the likelihood of bacterial phagocytosis (Lohinai et al., 2012).

There have been several studies in which distinct pathways for specific virulence mechanisms of bacterial species, (*Francisella*, *Legionella*, *Salmonella* spp. and *Shigella* spp.), have shown that polyamines were an essential requirement to establish an infection (Jelsbak et al., 2012; Nasrallah et al., 2011). Jelsbak et al (2012), demonstrated that by inhibiting polyamine synthesis pathways in *Shigella* spp, the virulence was severely limited by reducing bacterial ability to invade cells. This suggests that polyamines play a vital role in the modulation of the virulence of bacterial pathogens.

5.1 Polyamines and inflammation in periodontal disease

Inflammation is the physiological response to tissue damage which can result from microbial infection, cellular damage or response to toxic compounds (Chen et al., 2018). In the instance of acute inflammation, inflammatory cells such as leukocytes and lymphocytes are attracted to the site of the inflammatory event through signalling networks of cytokines, chemokines and growth factors (Babbar et al., 2007). Such cells contribute towards the breakdowns of tissues, whilst also maintaining defence against infections.

Key microbial makers of metabolic origin, which may provide crucial information on the periodontal inflammatory state of individuals, have been previously investigated as potential biomarkers (Sakanaka et al., 2017). PISA has been used to investigate disease associated metabolic signatures of periopathogens through a salivary metabolomics based approach (Kuboniwa et al., 2016). The polyamine cadaverine, has been specifically identified

to positivity correlate with PISA levels. Work by others has demonstrated the efficacy of cadaverine at reducing the secretion of bactericidal superoxide enzymes, resulting in the disruption of host immune response signalling pathways which in turn reduces leukocyte migration to the site of inflammation (Kang et al., 2007; Lohinai et al., 2012).

Some studies have demonstrated that putrescine and spermidine, which have been isolated from the GCF and inflamed periodontal pockets at concentrations above 1 mM and 200 μ M respectively, result in an increased disease stage of periodontitis. Furthermore, after treatment of the gingiva, the mean putrescine and spermine concentrations demonstrated significantly decreased concentrations. Such findings contribute to the theory of elevated polyamine levels in the GCF which coincide with increased periodontal disease severity for the patient. Polyamines in these instances are derived from oral bacteria and are released into the oral cavity following bacterial cell lysis as a response to the host immune system (Mariggio et al., 2004). Polymorphonuclear leukocytes, a type of immune cell which is responsible for controlling inflamed gingival sites are influenced by bacterially derived polyamines, and as a result are forced to undergo apoptosis. This results in the continuation of gingival inflammatory processes, since these leukocytes continually migrate to the local gingival crevice in response to a chemotactic releases of plaque bacterium thus, contributing towards prolonging the effects and damage of periodontal disease (deHart et al., 2008; Mariggio et al., 2004).

6.1 Polyamines and biomarkers of renal disease

Chronic kidney disease (CKD) is defined as the reduced kidney function that is present for three or more months and is determined using estimated glomerular filtration rate (GFR). The kidneys role is to filter the blood and metabolic waste whilst also altering the composition of fluids in the body. The loss off functional nephrons (basic structural unit of the kidney) results in a cascade of molecular and cellular events which may become pathological, and

develop into renal lesions (Rysz et al., 2017). Over recent years, CKD has become a major public health concern in both the developed and developing world (Fung and Kurella Tamura, 2016). CKD has a global affliction rate of up to 10% and is associated with a range of detrimental physiological and metabolic complications (Piccolli et al., 2017). It is characterised by the progressive destruction of the renal parenchyma, and along with the aforementioned loss of functional nephrons, ultimately leads to end stage renal failure (Viau et al., 2010). This progression is suggested to result from a perpetuating continuum of fibrosis, which remains active after the initial injury to the kidneys (Fogo, 2007). There have been numerous biomarkers identified for CKD, identifiable in a number of different locations and their effects on CKD have been suggested. Although CKD can be influenced by a range of conditions such as diabetes and high blood pressure and the effect of these comorbidities on CKD have been widely researched, much less research has been carried out to determine the influence of bacterially mediated progression of CKD (Thomas et al., 2008; Tuttle et al., 2019). Reported links between CKD and microorganisms isolated in distal locations have been studied, and one such correlation occurs between CKD and periodontitis (Lertpimonchai et al., 2019; Ariyamuthu et al., 2013). The potential systemic changes that periodontitis incurs are a result of dysbiosis of the oral microbiota, and the metastatic infection due to transient bacteria. In typical instances, the dissemination of oral microorganisms into the circulation result in no significant aftereffects other than short lived transient bacteraemia (Velzen et al., 1984). However, under favourable conditions the bacteria are able to localise and cause infection. The possibility of bacteria to undergo translocation from the oral cavity to distant body locations is one of the possible mechanisms in how oral microorganisms may contribute to systemic disease.

6.1.1 Biomarkers of chronic kidney disease

The estimation of kidney functionality has been previously conducted using biomarkers such as serum creatinine, elevated blood urea nitrogen levels and urine analysis (Rysz et al., 2017). However, biomarkers such as serum creatinine have been shown to possess low predictive values of CKD, thus efforts are being made into identifying new potential biomarkers for early CKD detection (Khan and Pandey, 2014). Within a clinical setting, GFR has been imperative in the diagnosis of CKD (Ruggenenti et al., 2012; Levey et al., 2014). GFR refers to the volume of fluid, which is filtered from the glomerular capillaries and into the Bowman's capsules as a function of time (Lopez-Giacoman and Madero, 2015). The GFR estimations are based on endogenous serum biomarkers such as serum creatinine or cystatin C. However, some studies suggest that biomarkers such as creatinine demonstrate suboptimal sensitivity and specificity in early CKD detection (Banda et al., 2020). In addition, factors such as age variations, sudden weight changes as well as current lack of standardisation of testing kits represent some of the issues with utilising serum creatinine as a CKD biomarker (Delanaye et al., 2014). Given the limitations of using serum creatinine as an indicator of kidney function decline, there has been an increase in the use of prediction equations using endogenous filtration biomarkers without the need for calculating renal clearance efficiency (Levey, 1990). Some studies suggest the effects of several alternate biomarkers for the detection and effective staging of CKD (Lopez-Giacoman and Madero, 2015).

6.1.2 Polyamines in chronic kidney disease

Since the 1970's, that the role of polyamines has been linked to CKD and it has been proposed that they may be influential in the pathogenesis of renal disorders (Macdougall, 2001). More recently, there has been an increase in the literature on the topic of polyamine-based CKD biomarkers (Igarashi et al., 2006; Saito et al., 1983; Sindhu, 2016; Goek et al.,

2013). Putrescine, a polyamine which is broken down into spermidine and spermine, was shown to be significantly elevated in patients with chronic renal failure (Saito et al., 1983), and it was postulated that such molecules may play an active role in the anaemia of end stage renal disease (Table 3), since polyamines such as putrescine have been shown to reduce the proliferation and maturation of erythroid cells (Kushner et al., 1991). Such studies suggest that the measurement of polyamines such as putrescine, may aid in determining the health of the kidneys in patients.

Table 3. The serum concentrations of putrescine, spermidine and spermine in normal and end stage renal disease patients.

Pre and Post CKD levels of Serum Polyamines	
Normal Subjects	Patients with ESRD
Putrescine 0.24 nmol/mL	Putrescine 0.51 nmol/mL
Spermine 0.20 nmol/mL	Spermine 0.05 nmol/mL
Spermidine N/A	Spermidine 0.34 nm/mL

559

560 It has been demonstrated that the addition of spermine and spermidine to *in vitro*
561 cultures of human KB and bovine BME-UV1 cells causes significant inhibition of cell growth,
562 potentially due to polyamine oxidation and breakdown into their respective components (Fusi et
563 al., 2008; Higgins et al., 1969). The concentrations of intracellular polyamines have been
564 shown to increase to millimolar levels, and during polyamine catabolism, the resultant H₂O₂
565 concentrations are sufficient to cause cellular oxidative damage (Murray-Stewart et al., 2016).
566 Furthermore, studies have demonstrated that there is an increase in the expression of polyamine
567 catabolic enzymes in distal organs, such as the liver in the event of kidney injury. Such
568 information supports the suggestion that disruption to the kidneys may have potential systemic
569 effects on distal organs via the upregulated metabolism of polyamines.

570

571 *6.1.3 Acrolein and polyamines as renal toxins*

572 In addition to the polyamines demonstrating toxic effects towards cell lines, a major
573 uremic toxin acrolein, derived from polyamine degradation, has been demonstrated to be in
574 high concentrations in those with chronic renal failure (Fig 5) (Sindhu, 2016). It was suggested
575 that the expressed levels of acrolein found in the patient plasma (170 µM/mL), were around 5
576 fold higher than in the plasma of healthy individuals (Igarashi et al., 2006). These
577 concentrations may be sufficient to cause cell damage, which may in turn further progress
578 kidney disease. The levels of spermine/spermidine-N¹-acetyltransferase (SSAT), an enzyme
579 which is essential for the breakdown of polyamines in serum, is also increased when the
580 kidneys are not functioning to their optimum capacity, such as in patients with kidney failure
581 or kidney injury (Zahedi et al., 2019). This action of polyamine degradation results in increased
582 levels of breakdown products which are rapidly excreted or oxidised by enzymes such as
583 putrescine oxidase, resulting in putrescine formation. This polyamine has been strongly

correlated with tissue damage in a large number of diseases (Minois et al., 2011). A rat perfusion model of ischemic reperfusion injury of the kidneys was used to measure the levels of putrescine, SSAT and putrescine oxidase. Such measurements demonstrated increased concentrations of upwards of 7.5 fold increases in the cortical collecting ducts of rat kidneys. The levels of SSAT peaked and remained unchanged after 12 h of reperfusion, indicating that the upregulation of SSAT can be utilised as a specific and sensitive biomarker for kidney injury (Zahedi et al., 2003). Thus, it has been hypothesised that the polyamines and their respective degradation enzymes may play a role in mediating kidney damage.

Figure 5. The changes in the polyamine levels as a result of chronic kidney disease. Putrescine levels are increase in kidney disease, which initially result in the increased formation of spermidine and spermidine. However, spermine and spermidine are rapidly broken down into

their oxidation products, namely acrolein. Thus, acrolein concentrations increase due to increased breakdown of polyamine.

7.1 Polyamines as biomarkers of human cancer

Cancer currently accounts for over one-sixth of global mortality rates (Bray et al., 2018). Over recent years, the identification and application of cancer biomarkers has become one the key foci of oncological research. Cancer biomarkers are described as biomolecules which are produced by either tumour cells, or by cells in response to a tumour (Goossens et al., 2015). Biomarkers of cancer must be able to provide prognostic, diagnostic or predictor values for patient outcomes (Nair et al., 2018). Despite the efforts made in biomarker research (over 1000 individual markers discovered), only a small number of markers (<25) have been approved for clinical use thus far (Vlachostergios and Faltas, 2019). A major issue is that although some biomarkers may demonstrate strong clinical validation, their ability to decisively contribute towards patient care is limited, outside of providing incremental, albeit clinically non essential information (Diamandis, 2012).

7.1.1 Polyamines as cancer biomarkers

The use of polyamines in the detection of cancer has been studied throughout the literature, since it has been suggested that their role in the pathogenesis of cancers such as prostate, colon and pancreatic, is apparent (Nowotarski et al., 2013; Soda, 2011; Damiani and Wallace, 2018). The first reported role of polyamines in cancer was documented by Russell and Snyder, (1968), whereby the increased levels of ornithine decarboxylase (ODC), a biosynthetic polyamine enzyme, was identified in various pathological cancers, such as liver and breast cancer (Russell and Snyder, 1968; Deng et al., 2008). When suppressed, the activity of ODC, the rate limiting enzyme for mammalian polyamine biosynthesis, showed inhibition

of colon carcinogenesis in cancer rodent models. Such a finding may suggest that the reduction in polyamine biosynthesis demonstrated a positive correlation in reducing the rates of colon cancer in rodents (Erdman et al., 1999).

In cancer, polyamine metabolism is frequently dysregulated, and with overall polyamine levels increased significantly, and this has been suggested that they are a necessity for tumour progression and transformation (Murray-Stewart et al., 2016). It has been further shown that rapid tumour growth has been associated with polyamine biosynthesis, since polyamines are upregulated in actively growing tumour cells as demonstrated by Khuhawar and Qureshi (2001), whereby, plasma putrescine levels were increased from 0.97 $\mu\text{g/ mL}$ to 1.65 $\mu\text{g/ mL}$ after a single day of measurements. Furthermore, work has shown that inhibition of the enzymes which regulate polyamine systems have resulted in the regression of tumour growth (Russell, 1977; Khuhawar and Qureshi, 2001). Previous works have hypothesised on the use of polyamines as potential cancer biomarkers, but low marker sensitivity in tissues has been a hindrance in these instances (Casero et al., 2018).

In the blood, the increased levels of polyamines directly reflect the exacerbated levels of polyamine synthesis, which exist as a result of cancer tissues augmenting the synthetic abilities of amine oxidase enzymes (Sun et al., 2017). Such is evident in instances of breast cancer whereby, increased proliferation and progression of breast cancer, in part, is stimulated by overexpression of polyamine synthesis. Breast cancer is one of the most common malignancies which affects women, in the western world (Torre et al., 2017). ODC, a polyamine decarboxylating enzyme, has been detected at increased levels in breast cancer (2.42 $\text{nmol CO}_2/\text{ hr g}$), in comparison to benign tumours (0.62 $\text{nmol CO}_2/\text{ hr g}$) (Cañizares et al., 1999). This suggests that increased polyamine levels may contribute towards the active disease and therefore such biomarkers in the blood may allow valuable diagnostic measurements for the differentiation between active and inactive cancers. Furthermore, ODC has been recognised

as in independent prognostic factor for localised breast cancer, and successful attempts at blocking polyamine receptors on ODC have been shown to provide a beneficial impact on the implications of breast cancer treatment (Jun et al., 2007).

7.1.2 Polyamine upregulation and detection in cancer

The amino acid derived polyamines (putrescine, spermine, spermidine and cadaverine), have been acknowledged as metabolites which are a necessity for cellular growth, interacting with negatively charged molecules such as DNA, RNA and phospholipids to facilitate biological processes such as transcription, translation and differentiation (Gerner and Meyskens, 2004; Mandal et al., 2013). With such a vital role in key molecular processes, it is possible for complications to occur, suggesting that polyamines may also play a role in the advancement of malignant states and tumorigenesis of actively replicating cells (Battaglia et al., 2014). The biosynthesis of polyamines is an upregulated process in all actively replicating cells, of which includes cancerous cells and the baseline values for healthy versus disease patients have been determined (Table 4) (Erdman et al., 1999).

Table 4. Levels of polyamines in the urine of patients with active cancer, and normal controls.

	Putrescine (µg/ mg)	Spermidine (µg/ mg)	Spermine (µg/ mg)
Control	2.1	1.2	0.04
Solid tumours	3.7	2.7	0.6
Haematological tumours	4.4	3.7	0.8

666

667 Patients with enhanced polyamine concentrations of spermidine and spermine (45.15
668 nmol/10¹⁰ red blood cells and 17.27 nmol/10¹⁰ red blood cells in the instance of pancreatic
669 cancer) in the blood have been shown to have more advanced disease states and more
670 unfavourable prognosis, comparative to those with lower polyamine levels (15.04 nmol/10¹⁰
671 red blood cells, and 8.82 nmol/10¹⁰ red blood cells respectively), regardless of the type of
672 malignancy (Uehara et al., 1980; Cipolla et al., 1994). Due to polyamines being vital for all
673 cell growth, it is possible that increases in polyamine synthesis as a result of cancer could reflect
674 enhanced tumour proliferation (Soda, 2011). Cancer cells with a greater capability to
675 synthesize polyamines are associated with an increased production of biomolecules such as
676 serine proteinase, matrix metalloproteinases, cathepsins and plasminogen activator, which can
677 degrade the surrounding tissues (Mason and Joyce, 2013). Furthermore, increased cancerous
678 tissues are able to produce elevated levels of vascular endothelial growth factors (VEGF),
679 enabling sufficient blood flow to the cancer tissues, which in turn increases their uptake of
680 polyamines, further augmenting cancerous cell migration (Auvinen et al., 1997; Ferrara, 2004).
681 Cancerous cells invade surrounding tissues through migrations using blood vessel in order to
682 establish secondary tumours (Soda, 2011). The overexpression of ODC and resultant increased
683 polyamine synthesis is suggested to increase the invasive properties of cancerous cells
684 therefore, it is possible that cancerous cells utilise polyamines as potential tumorigenesis
685 enhancing molecules to progress its diseases state (Kubota et al., 1997).

686

687 *7.1.3 Pancreatic cancer and its oral detection*

688 Pancreatic cancer continues to maintain the worst prognoses with a five-year survival
689 rate of only 9% (Ilic and Ilic, 2016). The poor prognosis rates alongside late presentation of
690 pancreatic cancer demonstrates the importance of early detection, thus aiming to minimise the

patient's risk of progressing to later stages of the disease (Asai et al., 2018). Upon initial diagnosis, 30% of patients present with a locally advanced tumour and 50% are diagnosed with pre-metastasized disease (Canto et al., 2012). Throughout the literature, work on novel metabolite based biomarkers have been investigated for detection of early pancreatic cancer (Mayerle et al., 2018; Lindahl et al., 2017). Techniques such as capillary electrophoresis – mass spectrometry based metabolomics have been utilised to analyse metabolites within the saliva of pancreatic cancer patients (Asai et al., 2018). The results discovered 24 markers which enabled discrete differentiation of pancreatic cancer from other cancer types. Amongst the elevated concentrations of the discriminatory metabolites, the polyamines, spermine and N_1 -acetyl spermine were found to be specific to pancreatic cancer at their respective increased concentrations, thus presenting a possible option as a potential indicator of pancreatic cancer.

In summary, the utilisation of polyamines and their respective breakdown enzymes provide an insight into the mechanisms in which they influence various cancer pathologies. Furthermore, biomarkers such as polyamines are easily assessable in patients' saliva and urine, suggesting their potential use as a non-invasive cancer biomarker. Although these markers demonstrate a means of rapid and non-invasive detection for a variety of cancers, their specificity towards a singular cancer remains to be elucidated. Thus, such biomarkers may be utilised in an initial assessment as an early indicator of potential disease, prior to the consideration of more invasive means of testing.

8.1 Polyamines, periodontitis and Alzheimer's disease

Alzheimer's disease (AD) is currently the most common neurodegenerative disease in humans and is amongst the most difficult to diagnose and treat at early stages (McGhee et al., 2014). Population studies using the Delphi consensus method, have shown that AD prevalence exceeds 50% in subjects aged 85+ years, indicating that this is a significant disease in older

people (Ferri et al., 2005). AD is characterised in a clinical setting through progressive memory and orientation loss, which include various other cognitive reductions. As it currently stands, only symptomatic AD treatments exist, such as anticholinesterase inhibitors and antiglutamaterics (Aisen et al., 2012). These methods of treatment involve dealing with the symptoms of AD as they manifest and do not directly influence the current disease state. In order to inhibit disease progression there must be interference with the pathogenic stages of the disease, thus disrupting disease evolution (Yiannopoulou and Papageorgiou, 2013).

In current practice, the presence of AD is confirmed through measurements of amyloid beta protein, tau protein and phospho-tau levels in the patient's cerebrospinal fluid (Fig 6) (Sharma and Singh, 2016; Galimberti and Scarpini, 2010). Neurofibrillary tangles are a fundamental pathological hallmark of AD and are aggregates of hyperphosphorylated tau protein (Serrano-Pozo et al., 2011). Tau protein is a microtubule-binding protein which is mainly expressed in neurons (Stoothoff and Johnson, 2005). The hyperphosphorylation of this protein is what is thought to be one of the causes of tau dysfunction, causing binding to the microtubules in the neurons, leading to the eventual disruption of the neuronal microtubules with subsequent impairment of axoplasmic flow and loss of neuronal connectivity (Grundke-Iqbal et al., 1986; Iqbal et al., 2000; Blennow and Zetterberg, 2018). Studies have suggested that the pathological severity of AD is at its greatest during the early stages of the disease. Due to the invasive nature of current diagnosis and treatment strategies, research aims are focusing towards alternate biomarker sources thus, aiming at slowing the progression of neurodegeneration (Nelson and Tabet, 2015; Thalhauser and Komarova, 2012; Folch et al., 2016).

Figure 6. Schematic of the neuron displaying intracellular neurofibrillary tangles and neuritic amyloid plaques. Key biomarkers which hallmark Alzheimer's disease, and the synaptic neurogranin biomarkers are demonstrated in their respective boxes.

8.1.1 Biomarkers for Alzheimer's disease

As it currently stands, there is no disease modifying treatment available for AD, but trials for pre-symptomatic and early symptomatic stages of the disease continue to occur (Cummings et al., 2018). In recent works, Aducanumab, a monoclonal antibody, was cancelled during trials following evidence that it could not effectively slow the decline in cognitive function through amyloid beta production and aggregation (Panza et al., 2019). Therefore, the requirement for sensitive, diagnostic biomarkers for AD are essential, allowing for potential early disease modifying intervention.

There have been a number of suggested biomarkers for AD that have been discovered in the cerebrospinal fluid (Niemantsverdriet et al., 2017; Blennow, 2004). However, blood-based biomarkers have gained very little attention in the literature, with no blood-based

screening tool developed for use to this date (Panza et al., 2019). Blood-based biomarker presentation offers a distinct advantage over conventional cerebrospinal fluid screens and neuroimaging modalities due to their advantages which include being cost effective, relatively less invasive and being able to provide an optimal starting location for further multistage assessment (O'Bryant et al., 2011). Due to recent advances in protein detection using mass spectrometry based approaches, there have been studies to define clinically relevant blood-based biomarkers (Long et al., 2016). Work by Ray et al (2007), identified 18 proteins in AD patient's plasma. These proteins allowed for differentiation between AD patients and healthy controls with a high specificity. Under further investigation, the same proteins were analysed, and it was shown that these proteins demonstrated diagnostic accuracy of 90% in identifying AD disease patients from healthy controls (Britschgi et al., 2011). With such findings, it may be possible to use blood-based biomarkers as predictors of AD. However, one issue arising from using this type of biomarkers is their specificity. Across multiple studies, large clinical cohorts were used in an attempt to identify markers of inflammation which are strongly associated with AD (Hu et al., 2012; Doecke et al., 2012; O'Bryant et al., 2011). Work carried out by the aforementioned researchers determined a high diagnostic accuracy with the 11 serum markers in test, including the polyamine cadaverine. The evidence suggested that cadaverine had some involvement in the inflammatory processes of AD, although there was no conclusive evidence of the exact role which cadaverine played. Therefore, the utilisation of new blood-based biomarkers has the potential to provide an alternative to conventional cerebral spinal fluid measurements.

8.1.2 Periodontitis influencing Alzheimer's disease

Periodontitis, due its potentially distal effects, has been suggested as major risk factor for the development of amyloid beta plaques and AD (Kamer et al., 2015). An observation

study consisting of 60 participants with mild to moderate AD was carried out by Ide et al (2016), and over a six month period, with comparisons made between the oral health status of the participant and cognitive decline. The presence of periodontal disease in these individuals demonstrated a 6 fold increase in the rate of cognitive decline, suggesting a correlation between the incidence of periodontal disease and the rate of cognitive decline in AD patients. In studies conducted by Hill et al (2014), and Kitazawa et al (2005), mice whom overexpressed the mutated tau precursor protein incurred significantly impaired cognitive function as a result of a *P. gingivalis* mediated oral infection. Furthermore, an increase in the deposition of plaques similar to those seen in AD patients demonstrated major oral bone loss. In addition to this, *P. gingivalis* lipopolysaccharides (LPS) have been detected within the human AD afflicted brain (Poole et al., 2013). These suggestions open up the hypothesis whereby, a *P. gingivalis* infection of the periodontium may play a potential role in the pathogenesis of AD (Singh et al., 2015).

9.1 Conclusion

Periodontitis, CKD, cancer and AD are all diseases which afflict a significant portion of the global population. Cadaverine represents a new potential biomarker for the detection of periodontal disease, with its presence in oral biofluids being correlated with the extent of periodontal tissue damage. It has been suggested for some time that there is a correlation between periodontitis and CKD, whereby systemic locations are influenced by periodontal bacteria or by their metabolites such as spermine, spermidine and acrolein, which have shown significant detrimental effects towards kidney cells. The polyamine, putrescine has demonstrated detrimental pathologies towards renal cells and has been suggested to act as uremic toxin alongside its degradation product, acrolein. Thus, such biomarkers may aid in current diagnostic strategies for CKD detection due to current markers such as creatinine

sometimes demonstrating low predictive values and significant variability between individuals. Spermidine and spermine demonstrate potential diagnostic biomarkers for the detection of cancer due to their significant involvement in the tumorigenesis of ongoing cancer pathologies. These polyamines are able to induce the production of vascular growth factors to enable increased blood flow to localised cancerous tissues. This in turn has suggested to increases polyamine uptake, augmenting cell proliferation and migration of oncogenic cells to distal sites. There is evidence to suggest the distal influences that periodontitis has on the systemic organs and tissues therefore, investigation into biomarkers which originate in the oral cavity could provide new means of disease detection. However, due to the limited number of studies investigating these associations in great depth, the evidence remains limited. Thus, further investigations are warranted to elucidate the reported interactions between these diseases.

The importance of polyamines in many molecular mechanisms in human disease is considerable. It is known that polyamines are in abundance in human biofluids during diseased states, and the evidence suggests strong correlations between the levels of polyamine and the extent or staging of a respective disease. Thus, it may be speculated that the utilisation of polyamine based biomarkers could provide the necessary diagnostic information in aiding efficient and rapid diagnosis of a disease.

For diseases such as periodontitis, traditional diagnostic means provide only information on the history of the disease, and not its current active state. Thus, a large amount of research is being undertaken to identify key biomarkers in oral fluids for not only oral infections, but also as a source of systemic diseases. Diseases such as prostate cancer is an example of a disease which effectively utilises biomarkers in their everyday screening and have shown a strong degree of functionality and repeatability. However, the uncertainty due to biomarker variability remains a challenge in the development of new biomarkers. Replicability and physiological variances between individual may hinder the effectiveness of a significant

portion of newly discovered biomarkers. Although these challenges do still remain, the utilisation of saliva and GCF as host media for biomarkers such as polyamines are very promising and warrant further investigation.

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Declaration of interest

No conflicts of interest were reported.

References

Aisen, P. S., Cummings, J., Schneider, L. S., 2012. Symptomatic and nonamyloid/tau based pharmacologic treatment for alzheimer disease. Cold Spring Harb Perspect Med. 2(3), a006395.

AlMoharib, H. S., AlMubarak, A., AlRowis, R., Geevarghese, A., Preethanath, R. S., Anil., S., 2014. Oral fluid based biomarkers in periodontal disease: part 1. Saliva. J Int Oral Health. 6(4), 95-103.

Ariyamuthu, V. K., Nolph, K. D., Ringdahl, B. E., 2013. Periodontal disease in chronic kidney disease and end-stage renal disease patients: a review. Cardiorenal Med. 3(1), 71-78.

Asai, Y., Itoi, T., Sugimoto, M., Sofuni, A., Tsuchiya, T., Tanaka, R., Tonozyuka, R., Honjo, M., 2018. Elevated polyamines in saliva of pancreatic cancer. Cancers. 10(2), 43.

856 Asikainen, S., Doğan, B., Turgut, Z., Paster, B. J., Bodur, A., Oscarsson, J., 2010. Specified
 857 species in gingival crevicular fluid predict bacterial diversity. PLoS One. 5(10), e13589.
 858

859 Auvinen, M., Laine, A., Paasinen-Sohns, A., Kangas, A., Kangas, L., Saksela, O., Andersson,
 860 L. C., Hölttä, E., 1997. Human ornithine decarboxylase-overproducing NIH3T3 cells induce
 861 rapidly growing, highly vascularized tumors in nude mice. Cancer Res. 57(14), 3016-3025.
 862

863 Babbar, N., Murray-Stewart, T., Casero, R. A., 2007. Inflammation and polyamine
 864 catabolism: the good, the bad and the ugly. Biochem Soc Trans. 35, 300-304.
 865

866 Banda, J., Duarte, R., Dix-Peek, T., Dickens, C., Manga, P. and Naicker, S., 2020.
 867 Biomarkers for diagnosis and prediction of outcomes in contrast-induced
 868 nephropathy. International Journal of Nephrology. 2020, 1-11.
 869

870 Barnes, V., Teles, R., Trivedi, H. T., Devizio, W., Xu, T., Mitchell, M., Milburn, M., Guo, L.,
 871 2009. Acceleration of purine degradation by periodontal diseases. Journal of dental research.
 872 88(9), 851-855.
 873

874 Barnes, V. M., Kennedy, A. D., Panagakos, F., Devizio, W., Trivedi, H. M., Jönsson, T.,
 875 Guo, L., Cervi, S., 2014. Global metabolomic analysis of human saliva and plasma from
 876 healthy and diabetic subjects, with and without periodontal disease. PLoS One. 9(8),
 877 e105181.
 878

879 Barros, S. P., Williams, R., Offenbacher, S., Morelli, T., 2016. Gingival crevicular fluid as a
 880 source of biomarkers for periodontitis. Periodontol 2000. 70(1), 53-64.

881

882 Battaglia, V., DeStefano Shields, C., Murray-Stewart, T., Casero, R. A., 2014. Polyamine
883 catabolism in carcinogenesis: potential targets for chemotherapy and chemoprevention.
884 Amino Acids. 46(3), 511-519.

885

886 Berezow, A. B., Darveau, R. P., 2011. Microbial shift and periodontitis. Periodontol 2000.
887 55(1), 36-47.

888

889 Blennow, K., 2004. Cerebrospinal fluid protein biomarkers for alzheimer's disease. NeuroRx.
890 1(2), 213-225.

891

892 Blennow, K., Zetterberg, H., 2018. Biomarkers for alzheimer's disease: current status and
893 prospects for the future. J Intern Med. 284(6), 643-663.

894

895 Bosshardt, D. D., 2018. The periodontal pocket: pathogenesis, histopathology and
896 consequences. Periodontol 2000. 76(1), 43-50.

897

898 Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., Jemal, A., 2018. Global
899 cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36
900 cancers in 185 countries. CA Cancer J Clin. 68(6), 394-424.

901

902 Britschgi, M., Rufibach, K., Huang, S. L., Clark, C. M., Kaye, J. A., Li, G., Peskind, E. R.,
903 Quinn, J. F., 2011. Modeling of pathological traits in alzheimer's disease based on systemic
904 extracellular signaling proteome. Mol Cell Proteomics. 10(10), M111.008862.

905

906 Canto, M. I., Hruban, R. H., Fishman, E. K., Kamel, I. R., Schulick, R., Zhang, Z., Topazian,
907 M., Takahashi, N., 2012. Frequent detection of pancreatic lesions in asymptomatic high-risk
908 individuals. *Gastroenterology*. 142(4), 796-804.
909

910 Casero, R. A., Murray Stewart, T., Pegg, A. E., 2018. Polyamine metabolism and cancer:
911 treatments, challenges and opportunities. *Nat Rev Cancer*. 18(11), 681-695.
912

913 Cañizares, F., Salinas, J., de las Heras, M., Diaz, J., Tovar, I., Martinez, P., Peñafiel, R.,
914 1999. Prognostic value of ornithine decarboxylase and polyamines in human breast cancer:
915 correlation with clinicopathologic parameters. *Clin Cancer Res*. 5(8), 2035-2041.
916

917 Cekici, A., Kantarci, A., Hasturk, H., Van Dyke, T. E., 2014. Inflammatory and immune
918 pathways in the pathogenesis of periodontal disease. *Periodontol 2000*. 64(1), 57-80.
919

920 Chen, L., Deng, H., Cui, H., Fang, J., Zuo, Z., Deng, J., Li, Y., Wang, X., Zhao, L., 2018.
921 Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget*. 9(6),
922 7204-7218.
923

924 Cho, M. I., Garant, P. R., 2000. Development and general structure of the periodontium.
925 *Periodontol 2000*. 24, 9-27.
926

927 Choong, M. K., Tsafnat, G., 2012. The implications of biomarker evidence for systematic
928 reviews. *BMC Med Res Methodol*. 12, 176.
929

930 Cipolla, B., Guille, F., Moulinoux, J. P., Bansard, J. Y., Roth, S., Staerman, F., Corbel, L.,
931 Quemener, V., 1994. Erythrocyte polyamines and prognosis in stage D2 prostatic carcinoma
932 patients. J Urol. 151(3), 629-633.
933

934 Collinson, P. O., 2013. Evaluating new diagnostic and prognostic biomarkers in
935 cardiovascular disease. Heart. 99(11), 757-758.
936

937 Cornejo Ulloa, P., van der Veen, M. H., Krom, B. P., 2019. Modulation of the oral
938 microbiome by the host to promote ecological balance. Odontology. 107(4), 437-448.
939

940 Correia-Neves, M., Fröberg, G., Korshun, L., Viegas, S., Vaz, P., Ramanlal, N., Bruchfeld, J.,
941 Hamasur, B., Brenan, P., Kallenius, G., 2019. Biomarkers for tuberculosis: the case for
942 lipoarabinomannan. ERJ Open Res. 5(1), 00115-2018.
943

944 Cortés-Vieyra, R., Rosales, C., Uribe-Querol, E., 2016. Neutrophil functions in periodontal
945 homeostasis. J Immunol Res. 2016, 1396106.
946

947 Cummings, J., Lee, G., Ritter, A., Zhong, K., 2018. Alzheimer's disease drug development
948 pipeline: 2018. Alzheimers Dement. 4, 195-214.
949

950 Damiani, E., Wallace, H. M., 2018. Polyamines and cancer. Methods Mol Biol. 1694, 469-
951 488.
952

953 Darveau, R. P., Arbabi, S., Garcia, I., Bainbridge, B., Maier, R. V., 2002. *Porphyromonas*
954 *gingivalis* lipopolysaccharide is both agonist and antagonist for p38 mitogen-activated protein
955 kinase activation. *Infect Immun.* 70(4), 1867-1873.

956

957 de Almeida, P. e. V., Grégio, A. M., Machado, M. A., de Lima, A. A., Azevedo, L. R., 2008.
958 Saliva composition and functions: a comprehensive review. *J Contemp Dent Pract.* 9(3), 72-
959 80.

960

961 de Pablo, P., Chapple, I. L., Buckley, C. D., Dietrich, T., 2009. Periodontitis in systemic
962 rheumatic diseases. *Nat Rev Rheumatol.* 5(4), 218-224.

963

964 deHart, G. W., Jin, T., McCloskey, D. E., Pegg, A. E., Sheppard, D., 2008. The $\alpha 9\beta 1$ integrin
965 enhances cell migration by polyamine-mediated modulation of an inward-rectifier potassium
966 channel. *In Proc Natl Acad Sci.* 105. 7188-7193.

967

968 Delanaye, P., Cavalier, E., Cristol, J., Delanghe, J., 2014. Calibration and precision of serum
969 creatinine and plasma cystatin C measurement: impact on the estimation of glomerular
970 filtration rate. *J Nephrol.* 27(5), 467-475.

971

972 Deng, W., Jiang, X., Mei, Y., Sun, J., Ma, R., Liu, X., Sun, H., Tian, H., Sun, X., 2008. Role
973 of ornithine decarboxylase in breast cancer. *Acta Biochim Biophys Sin.* 40(3), 235-243.

974

975 Deo, P., Deshmukh, R., 2019. Oral microbiome: Unveiling the fundamentals. *J Oral*
976 *Maxillofac Pathol.* 23(1), 122-128.

977

978 Diamandis, E. P., 2012. The failure of protein cancer biomarkers to reach the clinic: why, and
979 what can be done to address the problem?. BMC Medicine. 10(1), 1-5.

980

981 Dodds, M., Roland, S., Edgar, M., Thornhill, M., 2015. Saliva A review of its role in
982 maintaining oral health and preventing dental disease. BDJ Team. 2(1), 11-13.

983

984 Doecke, J. D., Laws, S. M., Faux, N. G., Wilson, W., Burnham, S. C., Lam, C. P., Mondal,
985 A., Bedo, J., Bush, J., Brown, B., Ruyck, K., Ellis, K., Fowler, C., Gupta, V., Head, R.,
986 Macaulay, L., Pertile, K., Rowe, C., Rembach, A., Rogrigues, M., Rumble, R., Szoeki, C.,
987 Taddei, K., Taddi, T., Trounson, B., Ames, D., Masters, C., Martins, R., 2012. Blood-based
988 protein biomarkers for diagnosis of Alzheimer disease. Arch Neurol. 69(10), 1318-1325.

989

990 Dye, B. A., Herrera-Abreu, M., Lerche-Sehm, J., Vlachoianis, C., Pikdoken, L., Pretzl, B.,
991 Schwartz, A., Papapanou, P. N., 2009. Serum antibodies to periodontal bacteria as diagnostic
992 markers of periodontitis. J Periodontol. 80(4), 634-647.

993

994 Erdman, S. H., Ignatenko, N. A., Powell, M. B., Blohm-Mangone, K. A., Holubec, H.,
995 Guillén-Rodriguez, J. M., Gerner, E. W., 1999. APC-dependent changes in expression of
996 genes influencing polyamine metabolism, and consequences for gastrointestinal
997 carcinogenesis, in the Min mouse. Carcinogenesis. 20(9), 1709-1713.

998

999 Ferrara, N., 2004. Vascular endothelial growth factor as a target for anticancer therapy.
1000 Oncologist. 9, 2-10.

1001

1002 Ferri, C. P., Prince, M., Brayne, C., Brodaty, H., Fratiglioni, L., Ganguli, M., Hall, K.,
1003 Hasegawa, K., 2005. Global prevalence of dementia: a Delphi consensus study. *Lancet*.
1004 366(9503), 2112-2117.
1005
1006 Fine, D., Mandel, I., 1986. Indicators of periodontal disease activity: an evaluation. *J Clin*
1007 *Periodontol.* 13(5), 533-546.
1008
1009 Fogo, A. B., 2007. Mechanisms of progression of chronic kidney disease. *Pediatr Nephrol*.
1010 22(12), 2011-2022.
1011
1012 Folch, J., Petrov, D., Ettcheto, M., Abad, S., Sánchez-López, E., García, M. L., Olloquequi,
1013 J., Beas-Zarate, C., 2016. Current research therapeutic strategies for alzheimer's disease
1014 treatment. *Neural Plast.* 2016, 8501693.
1015
1016 Fujita, T., Yoshimoto, T., Kajiya, M., Ouhara, K., Matsuda, S., Takemura, T., Akutagawa,
1017 K., Takeda, K., 2018. Regulation of defensive function on gingival epithelial cells can
1018 prevent periodontal disease. *Jpn Dent Sci Rev.* 54(2), 66-75.
1019
1020 Fung, E., Kurella Tamura, M., 2016. Epidemiology and public health concerns of ckd in
1021 older adults. *Adv Chronic Kidney Dis.* 23(1), 8-11.
1022
1023 Fusi, E., Baldi, A., Cheli, F., Rebucci, R., Ayuso, E., Sejrsen, K., Purup, S., 2008. Effects of
1024 putrescine, cadaverine, spermine, spermidine and β -phenylethylamine on cultured bovine
1025 mammary epithelial cells. *Ital J Anim Sci.* 7(2), 131-140.
1026

1027 Gadekar, N. B., Hosmani, J. V., Bhat, K. G., Kotrashetti, V. S., Nayak, R. S., Babji, D. V.,
 1028 Pattanshetty, S. M., Joshi, V. M., 2018. Detection of antibodies against aggregatibacter
 1029 actinomycetemcomitans in serum and saliva through ELISA in periodontally healthy
 1030 individuals and individuals with chronic periodontitis. *Microb Pathog.* 125, 438-442.
 1031
 1032 Galimberti, D., Scarpini, E., 2010. Treatment of alzheimer's disease: symptomatic and
 1033 disease-modifying approaches. *Curr Aging Sci.* 3(1), 46-56.
 1034
 1035 Gao, L., Xu, T., Huang, G., Jiang, S., Gu, Y., Chen, F., 2018. Oral microbiomes: more and
 1036 more importance in oral cavity and whole body. *Protein Cell.* 9(5), 488-500.
 1037
 1038 Gerner, E. W., Meyskens, F. L., 2004. Polyamines and cancer: old molecules, new
 1039 understanding. *Nat Rev Cancer.* 4(10), 781-792.
 1040
 1041 Ghallab, N. A., 2018. Diagnostic potential and future directions of biomarkers in gingival
 1042 crevicular fluid and saliva of periodontal diseases: Review of the current evidence. *Arch Oral*
 1043 *Biol.* 87, 115-124.
 1044
 1045 Goek, O. N., Prehn, C., Sekula, P., Römisch-Margl, W., Döring, A., Gieger, C., Heier, M.,
 1046 Koenig, W., Wang-Sattler, R., Illig, T., Suhre, K., Adamski, J., Köttgen, A., Meisinger, C.,
 1047 2013. Metabolites associate with kidney function decline and incident chronic kidney disease
 1048 in the general population. *Nephro Dial Trans.* 28(8), 2131-2138.
 1049
 1050 Goforth, J. B., Walter, N. E., Karatan, E., 2013. Effects of polyamines on vibrio cholerae
 1051 virulence properties. *PLoS One.* 8(4), e60765.

1052

1053 Goletti, D., Petruccioli, E., Joosten, S. A., Ottenhoff, T. H., 2016. Tuberculosis biomarkers:
1054 from diagnosis to protection. *Infect D Rep.* 8(2), 6568.

1055

1056 Gomez, B., Diaz, H., Carro, A., Benito, J., Mintegi, S., 2019. Performance of blood
1057 biomarkers to rule out invasive bacterial infection in febrile infants under 21 days old. *Archi*
1058 *Dis Child.* 104(6), 547-551.

1059

1060 Goossens, N., Nakagawa, S., Sun, X., Hoshida, Y., 2015. Cancer biomarker discovery and
1061 validation. *Trans Canc Res.* 4(3), 256-269.

1062

1063 Grundke-Iqbal, I., Iqbal, K., Tung, Y. C., Quinlan, M., Wisniewski, H. M., Binder, L. I.,
1064 1986. Abnormal phosphorylation of the microtubule-associated protein tau (tau) in Alzheimer
1065 cytoskeletal pathology. *Proc Nat Acad Sci.* 83(13), 4913-4917.

1066

1067 Guerra, P. R., Herrero-Fresno, A., Ladero, V., Redruello, B., Dos Santos, T. P., Spiegelhauer,
1068 M. R., Jelsbak, L., Olsen, J. E., 2018. Putrescine biosynthesis and export genes are essential
1069 for normal growth of avian pathogenic escherichia coli. *BMC Micro.* 18(1), 226.

1070

1071 Hajishengallis, G., 2015. Periodontitis: from microbial immune subversion to systemic
1072 inflammation. *Nat Rev Immuno.* 15(1), 30-44.

1073

1074 Hajishengallis, G., Lamont, R. J., 2012. Beyond the red complex and into more complexity:
1075 the polymicrobial synergy and dysbiosis (PSD) model of periodontal disease etiology. *Mol*
1076 *Oral Micro.* 27(6), 409-419.

1077

1078 Handa, A. K., Fatima, T., Mattoo, A. K., 2018. Polyamines: bio-molecules with diverse
1079 functions in plant and human health and disease. *Front Chem.* 6, 10.

1080

1081 Hernández, M., Dutzan, N., García-Sesnich, J., Abusleme, L., Dezerega, A., Silva, N.,
1082 González, F. E., Vernal, R., Sorsa, T., Gamonal, J., 2011. Host-pathogen interactions in
1083 progressive chronic periodontitis. *J Dent Res.* 90(10), 1164-1170.

1084

1085 Hickey, N. A., Shalamanova, L., Whitehead, K. A., Dempsey-Hibbert, N., van der Gast, C.,
1086 Taylor, R. L., 2020. Exploring the putative interactions between chronic kidney disease and
1087 chronic periodontitis. *Crit Rev Micro.* 46(1), 61-77.

1088

1089 Hienz, S. A., Paliwal, S., Ivanovski, S., 2015. Mechanisms of bone resorption in
1090 periodontitis. *J of Immu Res.* 615486.

1091

1092 Higgins, M. L., Tillman, M. C., Rupp, J. P., Leach, F. R., 1969. The effect of polyamines on
1093 cell culture cells. *J of Cell Phys.* 74(2), 149-154.

1094

1095 Hill, J., Clement, C., Pogue, A., Bhattacharjee, S., Zhao, Y., Lukiw, W., 2014. Pathogenic
1096 microbes, the microbiome, and alzheimer's disease (AD). *Front Aging Neurosci.* 6, 127-132.

1097

1098 Hu, W. T., Holtzman, D. M., Fagan, A. M., Shaw, L. M., Perrin, R., Arnold, S. E., Grossman,
1099 M., Xiong, C., Craig-Schapiro, R., Clark, C. M., Pickering, E., Kuhn, M., Chen, Y., Van
1100 Deerlin, V. M., McCluskey, L., Elman, L., Karlawish, J., Chen-Plotkin, A., Hurtig, H. I.,
1101 Siderowf, A., Swenson, F., M-Y Lee, V., Morris, C., Trojanowski, J. Q., Soares, H.,

1102 Alzheimer's Disease Neuroimaging Initiative., 2012. Plasma multianalyte profiling in mild
1103 cognitive impairment and Alzheimer disease. *Neuro*. 79(9), 897-905.
1104
1105 Humphrey, S. P., Williamson, R. T., 2001. A review of saliva: normal composition, flow, and
1106 function. *J Pros Dent*. 85(2), 162-169.
1107
1108 Hwang, H., Hwang, B. Y., Bueno, J., 2018. Biomarkers in infectious diseases. *Dis Mark*.
1109 2018, 8509127.
1110
1111 Ide, M., Harris, M., Stevens, A., Sussams, R., Hopkins, V., Culliford, D., Fuller, J., Ibbett, P.,
1112 Raybould, R., Thomas, R., Puenter, U., Teeling, J., Perry, V. H., Holmes, C., 2016.
1113 Periodontitis and cognitive decline in alzheimer's disease. *PLoS One*. 11(3), e0151081.
1114
1115 Igarashi, K., Kashiwagi, K., 2000. Polyamines: mysterious modulators of cellular functions.
1116 *Biochem Biophys Res Comms*. 271(3), 559-564.
1117
1118 Igarashi, K., Ueda, S., Yoshida, K., Kashiwagi, K., 2006. Polyamines in renal failure. *Amino*
1119 *Acids*. 31(4), 477-483.
1120
1121 Ilic, M., Ilic, I., 2016. Epidemiology of pancreatic cancer. *World J Gastroentero*. 22(44),
1122 9694-9705.
1123
1124 Iqbal, K., Alonso, A. D., Gondal, J. A., Gong, C. X., Haque, N., Khatoon, S., Sengupta, A.,
1125 Wang, J. Z., Grundke-Iqbal, I., 2000. Mechanism of neurofibrillary degeneration and
1126 pharmacologic therapeutic approach. *J Neural Transm Suppl*. 59, 213-222.

1127

1128 Jelsbak, L., Thomsen, L. E., Wallrodt, I., Jensen, P. R., Olsen, J. E., 2012. Polyamines are
1129 required for virulence in *Salmonella enterica* serovar typhimurium. *PLoS One*. 7(4), e36149.

1130

1131 Jun, J. Y., Griffith, J. W., Bruggeman, R., Washington, S., Demers, L. M., Verderame, M. F.,
1132 Manni, A., 2007. Effects of polyamine depletion by alpha-difluoromethylornithine on in vitro
1133 and in vivo biological properties of 4T1 murine mammary cancer cells. *Breast Cancer Res Tr*.
1134 105(1), 29-36.

1135

1136 Kamer, A. R., Pirraglia, E., Tsui, W., Rusinek, H., Vallabhajosula, S., Mosconi, L., Yi, L.,
1137 McHugh, P., Craig, R. G., Svetcov, S., Linker, R., Shi, C., Glodzik, L., Williams, S., Corby,
1138 P., Saxena, D., de Leon, M. J., 2015. Periodontal disease associates with higher brain amyloid
1139 load in normal elderly. *Neurobiol Aging*. 36(2), 627-633.

1140

1141 Kamio, Y., 1987. Structural specificity of diamines covalently linked to peptidoglycan for
1142 cell growth of *veillonella alcalescens* and *Selenomonas ruminantium*. *J Bacterio*. 169(10),
1143 4837-4840.

1144

1145 Kang, I. H., Kim, J. S., Kim, E. J., Lee, J. K., 2007. Cadaverine protects *vibrio vulnificus*
1146 from superoxide stress. *J Microbiol Biotech*. 17(1), 176-179.

1147

1148 Katz, J., Sambandam, V., Wu, J. H., Michalek, S. M., Balkovetz, D. F., 2000.
1149 Characterization of *porphyromonas gingivalis*-induced degradation of epithelial cell
1150 junctional complexes. *Infect Immun*. 68(3), 1441-1449.

1151

1152 Keesman, M., Aarts, H., Vermeent, S., Häfner, M., Papies, E. K., 2016. Consumption
1153 simulations induce salivation to food cues. *PLoS One*. 11(11), e0165449.
1154
1155 Kesavalu, L., Sathishkumar, S., Bakthavatchalu, V., Matthews, C., Dawson, D., Steffen, M.,
1156 Ebersole, J. L., 2007. Rat model of polymicrobial infection, immunity, and alveolar bone
1157 resorption in periodontal disease. *Infect Immu*. 75(4), 1704-1712.
1158
1159 Khan, Z., Pandey, M., 2014. Role of kidney biomarkers of chronic kidney disease: An
1160 update. *Saudi J Bio Sci*. 21(4), 294-299.
1161
1162 Khuhawar, M. Y., Qureshi, G. A., 2001. Polyamines as cancer markers: applicable separation
1163 methods. *J Chromato B Biomed Sci App*. 764(1-2), 385-407.
1164
1165 Khurshid, Z., Mali, M., Naseem, M., Najeeb, S., Zafar, M. S., 2017. Human gingival
1166 crevicular fluids (GCF) proteomics: an overview. *Dent J*. 5(1), 12.
1167
1168 Kilian, M., Chapple, I. L. C., Hannig, M., Marsh, P. D., Meuric, V., Pedersen, A. M. L.,
1169 Tonetti, M. S., Wade, W. G., Zaura, E., 2016. The oral microbiome – an update for oral
1170 healthcare professionals. *Brit Dent J*. 221(10), 657-666.
1171
1172 Kitazawa, M., Salvatore, O., Yamasaki, T., Green, K., LaFerla, F., 2005. Lipopolysaccharide-
1173 induced inflammation exacerbates tau pathology by a cyclin-dependent kinase 5-mediated
1174 pathway in a transgenic model of alzheimer's disease. *J Neurosci*. 25(39), 8843-8853.
1175

1176 Kuboniwa, M., Sakanaka, A., Hashino, E., Bamba, T., Fukusaki, E., Amano, A., 2016.
1177 prediction of periodontal inflammation via metabolic profiling of saliva. *J Dent Res.* 95(12),
1178 1381-1386.
1179
1180 Kubota, S., Kiyosawa, H., Nomura, Y., Yamada, T., Seyama, Y., 1997. Ornithine
1181 decarboxylase overexpression in mouse 10T1/2 fibroblasts: cellular transformation and
1182 invasion. *J Nat Cancer Institute.* 89(8), 567-571.
1183
1184 Kulic, L., Unschuld, P. G., 2016. Recent advances in cerebrospinal fluid biomarkers for the
1185 detection of preclinical Alzheimer's disease. *Curr Op Neuro.* 29(6), 749-755.
1186
1187 Kuramitsu, H. K., He, X., Lux, R., Anderson, M. H., Shi, W., 2007. Interspecies interactions
1188 within oral microbial communities. *Microb Mol Biol Rev.* 71(4), 653-670.
1189
1190 Kurihara, S., Oda, S., Kato, K., Kim, H. G., Koyanagi, T., Kumagai, H., Suzuki, H., 2005. A
1191 novel putrescine utilization pathway involves gamma-glutamylated intermediates of
1192 *Escherichia coli* K-12. *J Biol Chem.* 280(6), 4602-4608.
1193
1194 Kusano, T., Berberich, T., Tateda, C., Takahashi, Y., 2008. Polyamines: essential factors for
1195 growth and survival. *Planta.* 228(3), 367-381.
1196
1197 Kushner, D., Beckman, B., Nguyen, L., Chen, S., Della Santina, C., Husserl, F., Rice, J.,
1198 Fisher, J., 1991. Polyamines in the anemia of end-stage renal disease. *Kidney Int.* 39(4), 725-
1199 732.
1200

1201 Lamont, R. J., Hajishengallis, G., 2015. Polymicrobial synergy and dysbiosis in inflammatory
1202 disease. *Trend Mol Med.* 21(3), 172-183.
1203
1204 Lamster, I. B., 1997. Evaluation of components of gingival crevicular fluid as diagnostic
1205 tests. *Annals Periodont.* 2(1), 123-137.
1206
1207 Lamster, I. B., Ahlo, J. K., 2007. Analysis of gingival crevicular fluid as applied to the
1208 diagnosis of oral and systemic diseases. *Annal New York Acad Sci.* 1098, 216-229.
1209
1210 Lamster, I. B., Mandella, R. D., Zove, S. M., Harper, D. S., 1987. The polyamines putrescine,
1211 spermidine and spermine in human gingival crevicular fluid. *Archi Oral Bio.* 32(5), 329-333.
1212
1213 Laugisch, O., Johnen, A., Maldonado, A., Ehmke, B., Bürgin, W., Olsen, I., Potempa, J.,
1214 Sculean, A., 2018. Periodontal pathogens and associated intrathecal antibodies in early stages
1215 of alzheimer's disease. *J Alzheimers Dis.* 66(1) 105-114.
1216
1217 Lertpimonchai, A., Rattanasiri, S., Tamsailom, S., Champaiboon, C., Ingsathit, A.,
1218 Kitiyakara, C., Limpianunchai, A., Attia, J., 2019. Periodontitis as the risk factor of chronic
1219 kidney disease: mediation analysis. *J Clin Periodontol.* 46(6), 631-639.
1220
1221 Levey, A. S., 1990. Measurement of renal function in chronic renal disease. *Kidney Int.*
1222 38(1), 167-184.
1223
1224 Levey, A. S., Inker, L. A., Coresh, J., 2014. GFR estimation: from physiology to public
1225 health, *Am J Kidney Dis.* 63(5), 820-834.

1226

1227 Lindahl, A., Heuchel, R., Forshed, J., Lehtiö, J., Löhr, M., Nordström, A., 2017.

1228 Discrimination of pancreatic cancer and pancreatitis by LC-MS metabolomics.

1229 Metabolomics. 13(5), 61.

1230

1231 Lohinai, Z., Keremi, B., Szoko, E., Tabi, T., Szabo, C., Tulassay, Z., Levine, M., 2012.

1232 Bacterial lysine decarboxylase influences human dental biofilm lysine content, biofilm

1233 accumulation, and subclinical gingival inflammation. J Periodontol. 83(8), 1048-1056.

1234

1235 Long, J., Pan, G., Ifeakor, E., Belshaw, R., Li, X., 2016. Discovery of novel biomarkers for

1236 alzheimer's disease from blood. Dis Markers. 2016, 4250480.

1237

1238 Lopez-Giacoman, S., Madero, M., 2015. Biomarkers in chronic kidney disease, from kidney

1239 function to kidney damage. World J Nephrol. 4(1), 57-73.

1240

1241 Lowry, L. K., 1995. Role of biomarkers of exposure in the assessment of health risks.

1242 Toxicol Lett. 77(1-3), 31-38.

1243

1244 Lubell, Y., Althaus, T., 2017. Biomarker tests for bacterial infection-a costly wait for the holy

1245 grail. Lancet Infect Dis. 17(4), 369-370.

1246

1247 Lynge Pedersen, A. M., Belstrøm, D., 2019. The role of natural salivary defences in

1248 maintaining a healthy oral microbiota. J Dent. 80, 3-12.

1249

1250 López, N. J., 2000. Occurrence of actinobacillus actinomycetemcomitans, porphyromonas
1251 gingivalis, and prevotella intermedia in progressive adult periodontitis. J Periodontol. 71(6),
1252 948-954.
1253
1254 Macdougall, I. C., 2001. Role of uremic toxins in exacerbating anemia in renal failure.
1255 Kidney Int Suppl. 78, 67-72.
1256
1257 Mandal, S., Mandal, A., Johansson, H. E., Orjalo, A. V., Park, M. H., 2013. Depletion of
1258 cellular polyamines, spermidine and spermine, causes a total arrest in translation and growth
1259 in mammalian cells. Proc Natl Acad Sci. 110(6), 2169-2174.
1260
1261 Mariggiò, M. A., Vinella, A., Pasquetto, N., Curci, E., Cassano, A., Fumarulo, R., 2004. In
1262 vitro effects of polyamines on polymorphonuclear cell apoptosis and implications in the
1263 pathogenesis of periodontal disease. Immuno Immunotox. 26(1), 93-101.
1264
1265 Marsh, P. D., 2003. Are dental diseases examples of ecological catastrophes?. Microbiol.
1266 149, 279-294.
1267
1268 Marsh, P. D., 2009. Role of the oral microflora in health. Micro Ecol Health Dis. 12(3), 130-
1269 137.
1270
1271 Mason, S., Joyce, J., 2011. Proteolytic networks in cancer. Trends Cell Biol. 21(4), 228-237.
1272

1273 Mayerle, J., Kalthoff, H., Reszka, R., Kamlage, B., Peter, E., Schniewind, B., González
1274 Maldonado, S., Pilarsky, C., 2018. Metabolic biomarker signature to differentiate pancreatic
1275 ductal adenocarcinoma from chronic pancreatitis. *Gut*. 67(1), 128-137.
1276
1277 Mayeux, R., 2004. Biomarkers: potential uses and limitations. *NeuroRx*. 1(2), 182-188.
1278
1279 McGhee, D. J., Ritchie, C. W., Thompson, P. A., Wright, D. E., Zajicek, J. P., Counsell, C.
1280 E., 2014. A systematic review of biomarkers for disease progression in Alzheimer's disease.
1281 *PLoS One*. 9(2), e88854.
1282
1283 Miller-Fleming, L., Olin-Sandoval, V., Campbell, K., Ralser, M., 2015. Remaining Mysteries
1284 of Molecular Biology: The Role of Polyamines in the Cell. *J Mol Biol*. 427(21), 3389-3406.
1285
1286 Minois, N., Carmona-Gutierrez, D., Madeo, F., 2011. Polyamines in aging and disease.
1287 *Aging*. 3(8), 716-732.
1288
1289 Murray-Stewart, T. R., Woster, P. M., Casero, R. A., 2016. Targeting polyamine metabolism
1290 for cancer therapy and prevention. *Biochem J*. 473(19), 2937-2953.
1291
1292 Nair, M., Sandhu, S. S., Sharma, A. K., 2018. Cancer molecular markers: A guide to cancer
1293 detection and management. *Semin Cancer Biol*. 52(1), 39-55.
1294
1295 Nair, S., Faizuddin, M., Dharmapalan, J., 2014. Role of autoimmune responses in periodontal
1296 disease. *Autoimmune Dis*. 2014, 596824.
1297

1298 Nakajima, T., Katsumata, K., Kuwabara, H., Soya, R., Enomoto, M., Ishizaki, T., Tsuchida,
1299 A., Mori, M., 2018. Urinary polyamine biomarker panels with machine-learning
1300 differentiated colorectal cancers, benign disease, and healthy controls. *Int J Mol Sci.* 19(3),
1301 29518931.
1302
1303 Nakamya, M. F., Ayoola, M. B., Park, S., Shack, L. A., Swiatlo, E., Nanduri, B., 2018. The
1304 role of cadaverine synthesis on pneumococcal capsule and protein expression. *Med Sci.* 6(1),
1305 29351189.
1306
1307 Nasrallah, G. K., Riveroll, A. L., Chong, A., Murray, L. E., Lewis, P. J., Garduño, R. A.,
1308 2011. *Legionella pneumophila* requires polyamines for optimal intracellular growth. *J*
1309 *Bacteriol.* 193(17), 4346-4360.
1310
1311 Nazar Majeed, Z., Philip, K., Alabsi, A. M., Pushparajan, S., Swaminathan, D., 2016.
1312 Identification of gingival crevicular fluid sampling, analytical methods, and oral biomarkers
1313 for the diagnosis and monitoring of periodontal diseases: a systematic review. *Dis Markers.*
1314 2016, 1804727.
1315
1316 Nazir, M. A., 2017. Prevalence of periodontal disease, its association with systemic diseases
1317 and prevention. *Int J Health Sci.* 11(2), 72-80.
1318
1319 Nelson, L., Tabet, N., 2015. Slowing the progression of Alzheimer's disease; what works?.
1320 *Ageing Res Rev.* 23, 193-209.
1321

1322 Neyraud, E., Prinz, J., Dransfield, E., 2003. NaCl and sugar release, salivation and taste
1323 during mastication of salted chewing gum. *Physiol Behav.* 79(4) 731-737.
1324

1325 Niemantsverdriet, E., Valckx, S., Bjerke, M., Engelborghs, S., 2017. Alzheimer's disease
1326 CSF biomarkers: clinical indications and rational use. *Acta Neurol Belg.* 117(3), 591-602.
1327

1328 Nishihara, T., Koseki, T., 2004. Microbial etiology of periodontitis. *Periodontol 2000.* 36, 14-
1329 26.
1330

1331 Nowotarski, S. L., Woster, P. M., Casero, R. A., 2013. Polyamines and cancer: implications
1332 for chemotherapy and chemoprevention. *Expert Rev Mol Med.* 15, 23432972.
1333

1334 Nędzi-Góra, M., Kowalski, J., Górka, R., 2017. The Immune Response in Periodontal
1335 Tissues. *Arch Immunol Ther Exp.* 65(5), 421-429.
1336

1337 O'Bryant, S. E., Xiao, G., Barber, R., Huebinger, R., Wilhelmsen, K., Edwards, M., Graff-
1338 Radford, N., Doody, R., 2011. A blood-based screening tool for Alzheimer's disease that
1339 spans serum and plasma: findings from TARC and ADNI. *PLoS One.* 6(12), e28092.
1340

1341 Ozeki, M., Nozaki, T., Aoki, J., Bamba, T., Jensen, K. R., Murakami, S., Toyoda, M., 2016.
1342 Metabolomic analysis of gingival crevicular fluid using gas chromatography/mass
1343 spectrometry. *Mass Spectrom.* 5(1).
1344

1345 Panza, F., Lozupone, M., Dibello, V., Greco, A., Daniele, A., Seripa, D., Logroscino, G.,
1346 Imbimbo, B. P., 2019. Are antibodies directed against amyloid- β (A β) oligomers the last call
1347 for the A β hypothesis of Alzheimer's disease?. *Immunotherapy*. 11(1), 3-6.
1348
1349 Park, M. H., Igarashi, K., 2013. Polyamines and their metabolites as diagnostic markers of
1350 human diseases. *Biomol Ther*. 21(1), 1-9.
1351
1352 Patil, P. B., Patil, B. R., 2011. Saliva: A diagnostic biomarker of periodontal diseases. *J*
1353 *Indian Soc Periodontol*. 15(4), 310-317.
1354
1355 Patil, S., Rao, R. S., Sanketh, D. S., Amrutha, N., 2013. Microbial flora in oral diseases. *J*
1356 *Contemp Dent Pract*. 14(6), 1202-1208.
1357
1358 Pedersen, A. M., Bardow, A., Jensen, S. B., Nauntofte, B., 2002. Saliva and gastrointestinal
1359 functions of taste, mastication, swallowing and digestion. *Oral Dis*. 8(3), 117-129.
1360
1361 Pegg, A. E., 2016. Functions of polyamines in mammals. *J Biol Chem*. 291(29), 14904-
1362 14912.
1363
1364 Piccolli, A. P., Nascimento, M. M. D., Riella, M. C., 2017. Prevalence of chronic kidney
1365 disease in a population in southern Brazil. *J Bras Nefrol*. 39(4), 384-390.
1366
1367 Plombas, M., Gobert, B., De March, A. K., Sarda, M. N., Sixou, M., Béné, M. C., Miller, N.,
1368 Faure, G. C., 2002. Isotypic antibody response to plaque anaerobes in periodontal disease. *J*
1369 *Periodontol*. 73(12), 1507-1511.

1370

1371 Poole, S., Singhrao, S. K., Kesavalu, L., Curtis, M. A., Crean, S., 2013. Determining the
1372 presence of periodontopathic virulence factors in short-term postmortem alzheimer's disease
1373 brain tissue. *J Alzheimers Dis.* 36(4) 665-677.

1374

1375 Preshaw, P. M., 2015. Detection and diagnosis of periodontal conditions amenable to
1376 prevention. *BMC Oral Health.* 15, 5.

1377

1378 Qiu, X., Liu, C., Ye, Y., Li, H., Chen, Y., Fu, Y., Liu, Z., Huang, X., 2017. The diagnostic
1379 value of serum creatinine and cystatin c in evaluating glomerular filtration rate in patients
1380 with chronic kidney disease: a systematic literature review and meta-analysis. *Oncotarget.*
1381 8(42), 72985-72999.

1382

1383 Ray, S., Britschgi, M., Herbert, C., Takeda-Uchimura, Y., Boxer, A., Blennow, K., Friedman,
1384 L. F., Galasko, D. R., 2007. Classification and prediction of clinical Alzheimer's diagnosis
1385 based on plasma signaling proteins. *Nat Med.* 13(11), 1359-1362.

1386

1387 Ruggenti, P., Gaspari, F., Cannata, A., Carrara, F., Cella, C., Ferrari, S., Stucchi, N.,
1388 Prandini, S., 2012. Measuring and estimating GFR and treatment effect in ADPKD patients:
1389 results and implications of a longitudinal cohort study. *PLoS One.* 7(2), e2533.

1390

1391 Russell, D., Snyder, S. H., 1968. Amine synthesis in rapidly growing tissues: ornithine
1392 decarboxylase activity in regenerating rat liver, chick embryo, and various tumors. *Proc Natl*
1393 *Acad Sci.* 60(4), 1420-1427.

1394

1395 Russell, D. H., 1977. Clinical relevance of polyamines as biochemical markers of tumor
1396 kinetics. Clin Chem. 23(1), 22-27.
1397
1398 Rysz, J., Gluba-Brzózka, A., Franczyk, B., Jabłonowski, Z., Ciałkowska-Rysz, A., 2017.
1399 Novel biomarkers in the diagnosis of chronic kidney disease and the prediction of its
1400 outcome. Int J Mol Sci. 18(8), 28777303.
1401
1402 Saito, A., Takagi, T., Chung, T. G., Ohta, K., 1983. Serum levels of polyamines in patients
1403 with chronic renal failure. Kidney Int Suppl. 16, 234-237.
1404
1405 Sakanaka, A., Kuboniwa, M., Hashino, E., Bamba, T., Fukusaki, E., Amano, A., 2017.
1406 Distinct signatures of dental plaque metabolic byproducts dictated by periodontal
1407 inflammatory status. Sci Rep. 7(2). 42818.
1408
1409 Sarapur, S., Shilpashree, H. S., 2012. Salivary pacemakers: a review. Dent Res J. 9, 20-25.
1410
1411 Sauter, E. R., 2017. Reliable biomarkers to identify new and recurrent cancer. Eur J Breast
1412 Health. 13(4), 162-167.
1413
1414 Schatzle, M., Loe, H., Lang, N., Burgin, W., Anerud, A., Boysen, H., 2004. The clinical
1415 course of chronic periodontitis. J Clin Perio. 31(12), 1122-1127.
1416
1417 Selleck, M. J., Senthil, M., Wall, N. R., 2017. Making meaningful clinical use of biomarkers.
1418 Biomark Insights. 12, 28659713.
1419

1420 Serrano-Pozo, A., Frosch, M. P., Masliah, E., Hyman, B. T., 2011. Neuropathological
1421 alterations in alzheimer disease. Cold Spring Harb Perspect Med. 1(1), a006189.
1422

1423 Shah, P., Swiatlo, E., 2008. A multifaceted role for polyamines in bacterial pathogens. Mol
1424 Microbiol 68(1), 4-16.
1425

1426 Shaikh, H. F. M., Patil, S. H., Pangam, T. S., Rathod, K. V., 2018. Polymicrobial synergy and
1427 dysbiosis: An overview. J Indian Soc Periodontol. 22(2), 101-106.
1428

1429 Sharma, N., Singh, A. N., 2016. Exploring biomarkers for alzheimer's disease. J Clin Diagn
1430 Res. 10(7), 1-6.
1431

1432 Sindhu, K. K., 2016 Uremic toxins: some thoughts on acrolein and spermine. Ren Fail.
1433 38(10), 1755-1758.
1434

1435 Singhrao, S. K., Harding, A., Poole, S., Kesavalu, L., Crean, S., 2015. Porphyromonas
1436 gingivalis periodontal infection and its putative links with alzheimer's disease. Mediators
1437 Inflamm. 137357.
1438

1439 Socransky, S. S., 1979. Criteria for the infectious agents in dental caries and periodontal
1440 disease. J Clin Periodontol. 6(7), 16-21.
1441

1442 Socransky, S. S., Haffajee, A. D., Cugini, M. A., Smith, C., Kent, R. L., 1998. Microbial
1443 complexes in subgingival plaque. J Clin Periodontol. 25(2), 134-144.
1444

1445 Soda, K., 2011. The mechanisms by which polyamines accelerate tumor spread. J Exp Clin
1446 Cancer Res. 30, 95.
1447
1448 Srivastava, N., Nayak, P. A., Rana, S., 2017. Point of care- a novel approach to periodontal
1449 diagnosis-a review. J Clin Diagn Res. 11, 1-6.
1450
1451 Stathopoulou, P. G., Buduneli, N., Kinane, D. F., 2015. Systemic biomarkers for
1452 periodontitis. Current Oral Health Reports. 2(4), 218-226.
1453
1454 Stoothoff, W. H., Johnson, G. V., 2005. Tau phosphorylation: physiological and pathological
1455 consequences. Biochim Biophys Acta. 1739, 280-297.
1456
1457 Strimbu, K., Tavel, J. A., 2010. What are biomarkers?. Curr Opin HIV AIDS. 5(6), 463-466.
1458
1459 Subbarao, K. C., Nattuthurai, G. S., Sundararajan, S. K., Sujith, I., Joseph, J., Syedshah, Y.
1460 P., 2019. Gingival crevicular fluid: an overview. J Pharm Bioallied Sci. 11, 135-139.
1461
1462 Sun, W. Y., Choi, J., Cha, Y. J., Koo, J. S., 2017. Evaluation of the expression of amine
1463 oxidase proteins in breast cancer. Int J Mol Sci. 18(12), 29261141.
1464
1465 Suzuki, N., Yoneda, M., Hirofuji, T., 2013. Mixed red-complex bacterial infection in
1466 periodontitis. Int J Dent. 587279.
1467
1468 Sweeney, M. D., Sagare, A. P., Zlokovic, B. V., 2018, Blood-brain barrier breakdown in
1469 Alzheimer disease and other neurodegenerative disorders. Nat Rev Neurol. 14(3), 133-150.

1470

1471 Taba, M., Kinney, J., Kim, A. S., Giannobile, W. V., 2005, Diagnostic biomarkers for oral
1472 and periodontal diseases. *Dent Clin North Am.* 49(3), 551-571.

1473

1474 Tábi, T., Lohinai, Z., Pálfi, M., Levine, M., Szökő, É., 2008. CE–LIF determination of
1475 salivary cadaverine and lysine concentration ratio as an indicator of lysine decarboxylase
1476 enzyme activity. *Anal Bioanal Chem.* 391(2), 647-651.

1477

1478 Takahashi, K., Mooney, J., Frandsen, E. V., Kinane, D. F., 1997. IgG and IgA subclass
1479 mRNA-bearing plasma cells in periodontitis gingival tissue and immunoglobulin levels in the
1480 gingival crevicular fluid. *Clin Exp Immunol.* 107(1), 158-165.

1481

1482 Takahashi, T., Kakehi, J., 2010. Polyamines: ubiquitous polycations with unique roles in
1483 growth and stress responses. *Ann Bot.* 105(1), 1-6.

1484

1485 Tang, B. M., Shojaei, M., Parnell, G. P., Huang, S., Nalos, M., Teoh, S., O'Connor, K.,
1486 Schibeci, S., 2017. A novel immune biomarker. *Eur Respir J.* 49(6), 28619954.

1487

1488 Tarnanas, I., Tsolaki, A., Wiederhold, M., Wiederhold, B., Tsolaki, M., 2015. Five-year
1489 biomarker progression variability for Alzheimer's disease dementia prediction: Can a
1490 complex instrumental activities of daily living marker fill in the gaps?. *Alzheimers Dement.*
1491 1(4), 521-532.

1492

1493 Taylor, J. J., Preshaw, P. M., 2016. Gingival crevicular fluid and saliva. *Periodontol* 2000.
1494 70(1), 7-10.

1495

1496 Thalhauser, C. J., Komarova, N. L., 2012. Alzheimer's disease: rapid and slow progression. J
1497 R Soc Interface. 9(66), 119-126.

1498

1499 Thoden van Velzen, S. K., Abraham-Inpijn, L., Moorer, W. R., 1984. Plaque and systemic
1500 disease: a reappraisal of the focal infection concept. J Clin Periodontol. 11(4), 209-220.

1501

1502 Thomas, R., Kanso, A., Sedor, J., 2008. Chronic kidney disease and its complications. Prim
1503 Care. 35(2), 329-344.

1504

1505 Thyagarajan, B., Howard, A. G., Durazo-Arvizu, R., Eckfeldt, J. H., Gellman, M. D., Kim, R.
1506 S., Liu, K., Mendez, A. J., 2016. Analytical and biological variability in biomarker
1507 measurement in the hispanic community health study/study of latinos. Clin Chim Acta. 463,
1508 129-137.

1509

1510 Tofalo, R., Cocchi, S., Suzzi, G., 2019. Polyamines and gut microbiota. Front Nutr. 6, 16.

1511

1512 Torre, L. A., Islami, F., Siegel, R. L., Ward, E. M., Jemal, A., 2017. Global cancer in women:
1513 burden and trends. Cancer Epidemiol Biomarkers Prev. 26(4), 04, 444-457.

1514

1515 Tsuchida, S., Satoh, M., Takiwaki, M., Nomura, F., 2017. Ubiquitination in periodontal
1516 disease: a review. Int J Mol Sci. 18(7), 28698506.

1517

1518 Tuttle, K., Alicic, R., Duru, O., Jones, C., Daratha, K., Nicholas, S., McPherson, S.,
1519 Neumiller, J., Bell, D., Mangione, C. and Norris, K., 2019. Clinical characteristics of and risk

1520 factors for chronic kidney disease among adults and children. JAMA Netw Open. 2(12),
1521 e1918169.

1522

1523 Uehara, N., Shirakawa, S., Uchino, H., Saeki, Y., 1980. Elevated contents of spermidine and
1524 spermine in the erythrocytes of cancer patients. Cancer. 45(1), 108-111.

1525

1526 Urdea, M. S., Neuwald, P. D., Greenberg, B. L., Glick, M., Galloway, J., Williams, D.,
1527 Wong, D. T. 2011. Saliva, diagnostics, and dentistry. Adv Dent Res. 23(4), 353-359.

1528

1529 Viau, A., El Karoui, K., Laouari, D., Burtin, M., Nguyen, C., Mori, K., Pillebout, E., Berger,
1530 T., 2010. Lipocalin 2 is essential for chronic kidney disease progression in mice and humans.
1531 J Clin Invest. 120(11), 4065-4076.

1532

1533 Vinogradov, E., Perry, M. B., 2000. Structural analysis of the core region of
1534 lipopolysaccharides from *Proteus mirabilis* serotypes O6, O48 and O57. Eur J Biochem.
1535 267(8), 2439-2446.

1536

1537 Vlachostergios, P. J., Faltas, B. M., 2019. The molecular limitations of biomarker research in
1538 bladder cancer. World J Urol. 37(5), 837-848.

1539

1540 Wahid, A., Chaudhry, S., Ehsan, A., Butt, S., Ali Khan, A., 2013. Bidirectional relationship
1541 between chronic kidney disease & periodontal disease. Pak J Med Sci. 29(1), 211-215.

1542

1543 Wallis, R. S., Pai, M., Menzies, D., Doherty, T. M., Walzl, G., Perkins, M. D., Zumla, A.,
1544 2010. Biomarkers and diagnostics for tuberculosis: progress, needs, and translation into
1545 practice. *Lancet*. 375(9729), 1920-1937.
1546
1547 Walters, J., 1987. Polyamine analysis of human gingival crevicular fluid. *J Periodontal Res*,
1548 22(6), 522-523.
1549
1550 Walters, J., Miller, T., Cario, A., Beck, F., Marucha, P., 1995. Polyamines found in gingival
1551 fluid inhibit chemotaxis by human polymorphonuclear leukocytes in vitro. *J Perio*. 66(4),
1552 274-278.
1553
1554 Wang, G. P., 2015. Defining functional signatures of dysbiosis in periodontitis progression.
1555 *Genome Med*. 7(1), 40.
1556
1557 White, M. T., Xie, S. X., 2013. Adjustment for measurement error in evaluating diagnostic
1558 biomarkers by using an internal reliability sample. *Stat Med*. 32(27), 4709-4725.
1559
1560 Xu, X., Veenstra, T. D., 2008. Analysis of biofluids for biomarker research. *Proteomics Clin*
1561 *Appl*. 2(11), 1403-1412.
1562
1563 Yeh, C. Y., Adusumilli, R., Kullolli, M., Mallick, P., John, E. M., Pitteri, S. J., 2017.
1564 Assessing biological and technological variability in protein levels measured in pre-
1565 diagnostic plasma samples of women with breast cancer. *Biomark Res*. 5, 30.
1566

1567 Yiannopoulou, K. G., Papageorgiou, S. G., 2013. Current and future treatments for
1568 Alzheimer's disease. *Ther Adv Neurol Disord.* 6(1), 19-33.
1569
1570 Yven, C., Bonnet, L., Cormier, D., Monier, S., Mioche, L., 2006. Impaired mastication
1571 modifies the dynamics of bolus formation. *Eur J Oral Sci.* 114(3), 184-190.
1572
1573 Zahedi, K., Barone, S., Soleimani, M., 2019. Polyamine catabolism in acute kidney injury. *Int*
1574 *J Mol Sci.* 20(19), 31561575.
1575
1576 Zahedi, K., Wang, Z., Barone, S., Prada, A. E., Kelly, C. N., Casero, R. A., Yokota, N.,
1577 Porter, C. W., 2003. Expression of SSAT, a novel biomarker of tubular cell damage,
1578 increases in kidney ischemia-reperfusion injury. *Am J Physiol Renal Physiol.* 284(5), 1046-
1579 1055.
1580