

# The Importance of Saliva Total Protein and $\alpha$ -amylase on Cerebral Palsy Children

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## The Importance of Saliva Total Protein and $\alpha$ -amylase on Cerebral Palsy Children

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### Abstract

Cerebral Palsy (CP) Children have a prevalence of caries 59%, 54% Tooth erosion, bad Oral Hygiene (OH) and 52%-59% Malocclusion higher than general population (3,3% CP children in Indonesia). Because their limitations in motoric and intellectual, to maintain their oral health care require modify and different approaches. Saliva plays an important role of demineralization-rem mineralization, if there are any disorder will be potential for infectious disease (caries). Saliva is used as a tool to diagnose health and disease status. Changes in saliva flowrate interfere to protection function and total saliva protein and  $\alpha$ -amylase. Recent studies indicate that total salivary protein and  $\alpha$ -amylase is used as a biomarker for preventive measures and helps diagnose oral cavity disease (Caries Risk Assessment).

The authors would like to analyze the relationship between total salivary protein,  $\alpha$ -amylase and oral cavity conditions of CP children. Permanent damage to the Central Neuro System causes neuromuscular disorders. The disruption of the osmoreceptor function will reduce the sensitivity of Angio vasopressin, so that CP children experience hypohydration which results in decreased salivary flowrate. Changes in total protein and  $\alpha$ -amylase resulted in decreased salivary protective function and decreased oral conditions in CP children.

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### Introduction

<sup>1</sup> *Cerebral Palsy* (CP) describes a group of permanent disorders in the development of movement and posture that cause activity limitation, which is associated with non-progressive disorders that occur in the developing fetal or infant brain. CP motor disorders are often accompanied by impaired sensation, perception, cognition, communication, behavior, epilepsy, and secondary musculoskeletal problems.<sup>1</sup> Incidence of CP in America is 1-3 cases per 1000 individuals or

every 200 births, 1 child affected by this condition.<sup>2</sup> Dental health problems in CP children are not much different from normal children, where the incidence of dental caries, periodontal disease, and malocclusion is higher than the general population so that dental and oral health care for CP patients requires modifications and different treatment approaches from normal children due to limitations. which are owned. Disorders due to permanent damage to the motor center causing muscle weakness, stiffness, or paralysis, uncoordinated movements that require assistance from others to maintain their oral health.<sup>3,4</sup>

<sup>16</sup> Factors that can cause the high prevalence of dental and oral health problems in CP children are the consistency of the food consumed and carbohydrates amount that hit the teeth per day, difficulty chewing and swallowing food or drink due to oromotor dysfunction, difficulty in cleaning the oral cavity due to the biting reflex, long-term use of drugs with potential for decreased salivary

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secretion (xerostomia) and problems associated with dental management.<sup>5,6</sup> The important role of saliva in the balance of demineralization and remineralization of enamel in the oral environment has the potential to become caries where saliva has been studied as a tool for diagnosing health conditions and disease status.<sup>7,8</sup> The condition of hypohydration in CP children due to lack of water intake caused by CP children is very dependent on the initiative of caregivers or parents to provide water, as well as reduced swallowing ability due to oromotor dysfunction. In addition, the hypohydration condition of CP children is also caused by a disturbance in the hypothalamus which results in an electrolyte imbalance in the body and reduces the sensitivity of CP children, against thirst.<sup>9,10,11</sup>

Salivary secretion is related to the hydration conditions of a person's body and plays an important role in oral health because of its protective function and as a mechanical cleansing to help remove food debris where microorganisms thrive and hypohydration conditions cause salivary secretion to decrease as indicated by a decrease in saliva flowrate.<sup>2,11,12</sup> CP children with hypohydration conditions decreased salivary secretion so that they had a higher risk of caries activity and oral cavity disease, this was reported in a previous study which stated that there was a change in saliva flow rate and saliva biochemical parameters in the whole saliva of children with CP. Variations in the concentration of electrolyte ions, reduction and antimicrobial activity of a digestive enzyme amylase and peroxidase, sialic acid increased, decreased saliva flowrate, pH and buffer capacity, an increase in total protein and salivary osmolality<sup>13,14</sup> and strengthened by the research of Izzati, R. 2018, which states that the value of the osmolality of saliva in children is higher CP and salivary electrolyte level is low compared to normal children.<sup>15</sup> Therefore, this paper was conducted to analyze the relationship between the content of non-immune salivary enzymes, namely total protein and amylase, on the high prevalence of dental caries in children with CP.

## Review

Risk factors that can cause CP include prenatal hypoxia, metabolic and metabolic disorders, multiple pregnancies, intrauterine

infection, thrombophilic disorders, teratogenic exposure, chorioamnionitis, maternal fever, exposure to toxins, brain structural malformations, intrauterine growth restriction, trauma, abdomen, and vascular disorders. Perinatal conditions include asphyxia, preterm delivery (<32 weeks or <2500 grams), blood incompatibility, infection, abnormal fetal presentation, placental abruption, and labor. While postnatal conditions can occur asphyxia, seizures in the postnatal period, cerebral infarction, hyperbilirubinemia, sepsis, respiratory distress syndrome, chronic lung disease, meningitis, postnatal steroids, intraventricular hemorrhage, periventricular leukomalacia, infant shock, and head injury. Two patients with CP type the same person can show very different symptoms.<sup>2,16</sup>

Oral manifestations were seen to be more severe in CP children compared to the normal pediatric population, including periodontal disease, caries, malocclusion, bruxism, abrasion, and trauma due to poor oral hygiene and difficulty brushing or *flossing*, difficulty chewing, swallowing, and tending to eat soft foods. sweet and easy to swallow and contain high carbohydrates and long-term consumption of the drug phenytoin to control seizure activity will cause gingival hyperplasia. Inharmonious relationship between intraoral and perioral muscles in CP children, such as the uncoordinated and uncontrolled jaw, lip, and tongue movements. This can cause disturbances in chewing and swallowing, *drooling* saliva, *tongue thrusting*, speech disorders, temporomandibular joint disorders, and frequent trauma in children with CP, especially in the maxillary anterior teeth associated with a tendency to fall, along with reduced extensor reflexes, and presence of protrusion of maxillary anterior teeth.<sup>2,17,18</sup>

A study recent of saliva as a biological matrix has been identified as an important initiative in the research, which is used as a biomarker for the prevention and diagnosis of disease. In particular, saliva is a reservoir that is rich in proteins and peptides are different and can be identified by the latest advances in molecular biology, particularly in targeted proteomics technology and the results are not biased.<sup>19</sup> Saliva is the first line of defense against oxidative stress (OE), Reactive Oxygen Species (ROS), and free radicals when the imbalance

between ROS systemic manifestations has an involvement in the pathogenesis of more than 100 conditions of pathogens and free radicals in the theory of antiaging.<sup>20</sup>

It was reported in a previous study that there was a change in *salivary flow rate* and salivary biochemical parameters in the *whole saliva* of CP children. Variations in the concentration of sodium ion (Na) and potassium (K), the increase in total protein, reduced activity of digestive enzymes and antimicrobial-amylase and peroxidase, increasing the sialic acid, decreased *saliva* flowrate, pH, and buffer capacity, electrolyte concentration, and osmolality of saliva thus lowering protective function<sup>12,20</sup>. Where it can increase the risk of disease in the oral cavity in children CP is a high incidence of caries and periodontal disease their peningktan.<sup>19</sup>

According to previous studies mentioned that *salivary flow rate* has an influence on a person's status in the oral cavity, where the decline may increase the risk of caries and periodontal disease<sup>18</sup>. Strengthened by research by Santos, 2007 which states that there is a decrease in *salivary flow rate* 44% in children with CP, where boys have lower scores than girls when compared to normal children as controls. In addition, the total protein increase was 122% higher in CP children compared to normal children, wherein boys it was higher than in girls.<sup>19</sup>

The high value of total protein can be associated with a decrease in *salivary flow rate* in CP children. Human saliva is rich in proteins and peptides, more than 3652 proteins and peptides 12 562, nearly 51% protein and 79% of the peptides contained in plasma.<sup>17</sup> Salivary glycoproteins play an important role in mucosal defense and maintain mucosal surfaces with their viscoelastic properties, especially salivary mucin which contains complex carbohydrates and salivary proteins which have antimicrobial activity. So, knowledge about the composition of protein and carbohydrates is also needed.<sup>20</sup>

One of the non-immunological salivary proteins is  $\alpha$ -amylase<sup>21</sup>, where  $\alpha$ -amylase is a calcium-containing metalloenzyme that can hydrolyze 1,4 glycosidic bonds of several glycans such as carbohydrates or starch (amylopectin) to produce oligosaccharides (dextrin), disaccharides (maltose, isomaltose), and monosaccharides (glucose). This enzyme is

<sup>18</sup> involved in the initiation of starch digestion in the oral cavity and its activity varies from person to person.  $\alpha$ -amylase is one of the major salivary proteins that appear as a total isoenzyme.  $\alpha$ -amylase accounts for 10-20% of the total protein produced by the salivary glands and is an enzyme that is widely found in saliva because it is an innate defense system of the oral mucosa.<sup>11,22</sup>

Research of Leite, et al. in 2013 reported that  $\alpha$ -amylase salivary CP children showed a decrease in concentration and secretion while the activity was not a significant difference, it is related to the concentration of total protein and saliva flowrate.<sup>11</sup> The concentration of  $\alpha$ -amylase increases with an increase in saliva flow rate and has antimicrobial properties that prevent the adhesion of bacteria to the teeth and mucosa.<sup>23</sup> This is reinforced by the study of Santos, 2007 which stated that salivary  $\alpha$ -amylase showed a decrease of about 60% in boys and 69% in girls with CP compared to normal children as controls.<sup>19</sup>  $\alpha$ -amylase shows a specific bond with the presence of attraction with microorganisms (cariogenic and periodontopathogen) forming agglomerates that are easily soluble by saliva during swallowing which results in an acidic atmosphere in the stomach.<sup>11</sup> It is strongly associated with the prevalence of caries and periodontal disease are higher in children CP, which is supported by a cross-sectional study Wyne, et al. in 2017 mentions children with CP have oral hygiene who poor and high caries figure<sup>24</sup>. In addition, the results of a study by Subramain et al. in 2010 stated that the prevalence of caries in the permanent teeth of CP children was higher than normal children, while caries in the primary teeth of CP and normal children had the same high value.<sup>6</sup>

Herdani, T. in 2018 stated that children with CP in Surabaya showed a high def-t/DMF-T value and was influenced by the age factor<sup>25</sup>. Recent studies have shown that children with CP have more acidic saliva with a higher dental plaque index, which is positively correlated with caries activity, is associated with high levels of RS (Reactive Species) and lipid peroxidation, exhibits greater oxidative damage and the presence of a defense system. Endogenous antioxidants, specifically related to caries activity and found lower salivary pH, greater accumulation of bacterial biofilm and salivary oxidation imbalance are factors that favor the

development of oral cavity disease in individuals with.<sup>26</sup>

## Discussion

Children with CP experience permanent damage to the central nervous system (*Central Neuro System/ CNS*), namely the brain and spinal cord, where this will affect the work system of the *Autonomic Nervous System (ANS)* and *Peripheral Neuro System (PNS)*, thus affecting the work of the system. sympathetic and parasympathetic nerves and interfere with motor function. These neuromotor disorders cause CP children to be unable to perform movements or activities (kinesthetic) like children of their age, in addition to oromotor disorders which result in difficulty in chewing, swallowing food and drinks. This results in a condition of *dysphagia* that affects the type of food consumption in CP children who consume more soft and liquid foods.

The presence of muscle stiffness or spasms that occur in CP children requires them to routinely take anti-convulsant drugs that are high in sugar content, while the side effect of consuming these drugs is an *enlargement of the gingiva* which can accelerate the process of pellicle plaque formation and make it difficult to clean it. Other manifestations are the habit of breathing through the mouth which causes cleaning *dry mouth* so that it affects the process of their *self-teeth*, as well as the difficulty of CP children in brushing their teeth due to the biting reflex. an imbalance of demineralization and remineralization that can lead to an increased risk of caries and periodontal disease.

Neuromotor disorders in CP children cause the *Somatic Nervous System (SNS)* to be disrupted, thus disrupting the function of osmoreceptors in the hypothalamus. This affects the regulation of secretion *arginine vasopressin (AVP)* which regulates thirst, resulting in an electrolyte imbalance in the body and decreased sensitivity or sensitivity of CP children to thirst.<sup>7,8,9</sup> Therefore, *intake* fluid decreases and causes CP children to experience conditions hypohydration, this is supported by research by Komatsu, et.al which states that CP children experience conditions hypohydration.<sup>27</sup> On the other hand cause dysfunction oromotor heavily dependent child or parent caregiver initiatives to meet fluid needs.<sup>3,4</sup>

Children with CP with hypohydration have decreased salivary secretion so they have a higher risk of caries activity.<sup>17</sup> Salivary secretion is important for oral health because of its protective function and mechanical cleansing and normal salivary secretion can help remove food debris where microorganisms thrive. Hypohydration causes a decrease in salivary secretion as indicated by a decrease in saliva flow rate and an increase in total protein, this is in line with the ability of the pH buffer to decrease and an increase in osmolality. This will affect the balance of the demineralization and remineralization processes on the enamel surface and affect the ability to eliminate bacteria in the oral cavity resulting in an increased incidence of caries and periodontal disease.<sup>2,9,10</sup>

ANS disorders in children with CP will affect the work of the sympathetic and parasympathetic nerves in the brain stem, where this will affect the electrolyte level of the acinar cells of the salivary glands in the process of primary salivary secretion, thus affecting the reabsorption of electrolytes in the secondary secretory process by the parotid salivary glands which will be excreted through the ducts parotid (*Stensen's duct*).<sup>23,28</sup> The presence of neuromuscular abnormalities in children with CP also affects the volume of saliva, this is due to reduced mechanical stimulation from the movement of orofacial muscles in the masticatory process. In addition, the process of salivary secretion is also influenced by the saliva flowrate, which in children with CP has decreased, causing an increase in the content of salivary organic matter, one of which is in the form of protein.

Salivary flow is controlled by the autonomic nervous system, where stimulation of the parasympathetic nerves results in a higher flow rate, whereas stimulation of the sympathetic nervous system produces a smaller flow but is richer in proteins and peptides. This stimulation gives a clear difference in the description of saliva proteome and also in the relative amounts of specific proteins that were detected.<sup>16,29</sup> Hypo saliva and mucosal moisture correlate with protein concentration, whereas an increase in protein concentration is to be affected by a decrease in salivary volume.<sup>16</sup> Children with CP are hypohydration so that they experience a decrease in saliva volume which causes an increase in the total protein value.

The increase in the total value of protein

and carbohydrates can be influenced by the minor salivary glands found on the mucosal surface of the oral cavity (residual saliva/RS).<sup>19</sup> Salivary proteins play a role in oral pathology, where salivary proteins can provide a cytoprotective function in many oral diseases and at the same time can contribute to the process of inflammation, infection, and even oral cancer. In this sense, the salivary proteome plays an important role in the homeostasis of the oral cavity, the presence of an imbalance in the immunological and nonimmunological salivary defense systems that can lead to various possible mechanisms that lead to oral pathology. An increase in the total protein value in CP children results in an imbalance in the oral defense system and decreases its cytoprotective function so that it can affect the prevalence of caries and periodontal disease in CP children.

Proteins having antimicrobial activity are secreted by the salivary glands, as the first line of defense against infection and disease by interfering with the adhesion of microorganisms to the mucosa.<sup>16</sup> Mucin on mucosal surfaces of the oral cavity has several functions, among others act as a permeability barrier to tissue damage, lubrication or lubrication of oral mucosal surface, concentrating antimicrobial molecules to the surface of the oral mucosa and modulate the colonization of bacteria, fungi, and viruses. The non-covalent interaction of salivary mucin with other salivary molecules can be interpreted as a mechanism by which salivary proteins are concentrated on the mucosal surface of the oral cavity. It has been reported that salivary mucin undergoes non-covalent interactions with proline-rich proteins, sIgA,  $\alpha$ -amylase, lysozyme, cystatin, statherin, histatin, and lactoferrin.<sup>19</sup>

Quantitatively important proteins are  $\alpha$ -amylase, a protein-rich in proline, mucin, and immunoglobulin. Total salivary protein contains enzymes, mucins, immunoglobulins, and others, where one of the enzymes that have an antimicrobial function is  $\alpha$ -amylase.<sup>30</sup>  $\alpha$ -amylase serves as a catalyst in the process glucokinase, namely converting carbohydrates (starches) into glucose or maltose is used as a provider of nutrients for streptococcus bacteria colonizes and metabolism in biofilm formation or pellicle plaque. In addition,  $\alpha$ -amylase functions as a receptor for microorganisms with specific binding (adhesion) to the tooth surface and functions as an antimicrobial enzyme of first defense in the

prevention of infections and oral diseases by blocking the attachment of pathogenic microbes to enamel surfaces with a pH of 6.5-7, as well as several studies have shown that salivary  $\alpha$ -amylase can be used as a marker of stress secreted by the salivary glands. So that  $\alpha$ -amylase can control biofilm adhesion on the tooth and mucosal surfaces.<sup>11,18,28</sup>

Salivary  $\alpha$ -amylase is considered an important enzyme in saliva, especially *Whole saliva* produced by the parotid gland. Saliva is mostly produced by serous acinar cells and its secretion is under sympathetic-parasympathetic control, whereas salivary  $\alpha$ -amylase is secreted from the parotid gland under  $\beta$ -adrenergic stimulation. Human studies have shown that the autonomic nervous system (ANS) plays an important role in the secretion of  $\alpha$ -amylase and involves both  $\alpha$  and  $\beta$ -Adrenergic mechanisms.<sup>29-31</sup>

ANS disorders in children with CP cause damage to the parasympathetic sympathetic nerves which will disrupt the release of Norepinephrine (NE) at the nerve endings of the salivary glands, so that the concentration of norepinephrine decreases. This causes  $\beta$ -adrenoreceptors in salivary glands, glandular ducts, and vascular beds unable to bind to NE, thus affecting the secretion of  $\alpha$ -amylase to decrease.<sup>27,29,32</sup> A decrease in  $\alpha$ -amylase results in a decrease in its function as an antimicrobial enzyme and interferes with the glucokinase process, resulting in an imbalance of demineralization-remineralization in the long term which will increase the incidence of dental caries and periodontal disease.

Previous research stated that  $\alpha$ -amylase increased when the condition was relaxed, while the condition of CP children who experienced muscle stiffness caused a decrease in  $\alpha$ -amylase concentration. An increase in the concentration of  $\alpha$ -amylase is characterized by Autonomic Neuronal Activation (ANS), as evidenced by research that levels of  $\alpha$ -amylase increase in response to physical stressors or physical activity. Meanwhile, CP children have ANS nervous disorders and limited physical activity, so they experience a decrease in  $\alpha$ -amylase concentration.<sup>33</sup> Reinforced by research from Leite, et al. 2013 reported that salivary  $\alpha$ -amylase in children with CP showed a decrease in concentration and secretion, while there was

no significant difference in activity, this was related to total protein concentration and saliva flowrate,<sup>11</sup> the concentration of  $\alpha$ -amylase increased with It increases the salivary flow rate and has antimicrobial properties that prevent the adhesion of bacteria to the teeth and mucosa.<sup>22</sup>

Research in molecular biology states that  $\alpha$ -amylase is also used as a biomarker to identify several diseases in the oral cavity, including *Recurrent Aphthous stomatitis* (RAS) and Burning mouth syndrome (Glossodynia).<sup>16</sup> From these data, it can be correlated that a decrease in  $\alpha$ -amylase salivary affects the incidence and prevalence of caries and diseases in the oral cavity of children with CP. It is corroborated by several studies which state that the prevalence of dental caries and periodontal disease in children with CP is higher than in normal children.

## Conclusions

Changes in total protein and  $\alpha$ -amylase resulted in decreased salivary protective function and decreased oral conditions in CP children.

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## Declaration of Interest

The authors declare that there is no conflict of interest.

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