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Analysis of Intracellular Enzymes in Saliva of Patients with Aggressive Periodontitis

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SUMMARY

Introduction Aggressive periodontitis is characterized by rapid destruction of periodontal tissue. It is more commonly found in young adults. Analysis of biochemical markers in saliva is very important to monitor the level of periodontal tissue destruction. The aim of this study was to analyze the intracellular enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in the saliva of patients with aggressive periodontitis.
Material and Methods The study included 22 patients with aggressive periodontitis and 28 healthy subjects (control group). Enzyme activity was measured in mixed non-stimulated saliva using kinetic methods in a spectrophotometer and expressed in international units per litre (U/L).
Results The activity of enzymes ALT and ALP was significantly higher in saliva of patients with aggressive periodontitis compared to healthy subjects, while AST activity was not significantly different. Also, there was no correlation between the activity of intracellular enzymes in saliva and clinical parameters such as gingival index, bleeding index, probing depth in patients with aggressive periodontitis.
Conclusion Obtained results indicate that salivary enzymes AST, ALT and ALP can be used as biochemical markers to aid in diagnosis of aggressive periodontitis.
Keywords: aggressive periodontitis; ALP; ALT; AST; intracellular enzymes; saliva

INTRODUCTION

Aggressive periodontitis is a disease associated with intense destruction of periodontal tissue. Diagnosis is based on clinical (plaque index, gingival index, probing depth, bleeding index, level of epithelial attachment) and radiographic parameters (degree of alveolar bone loss) [1, 2]. Clinical measurements are useful in the diagnosis of periodontitis, but they provide limited information regarding subclinical form of the disease, prognosis and treatment efficacy. The analysis of biochemical composition of saliva may be of value as an additional diagnostic tool.

Saliva is a secretion with complex structure and specific role. The analysis of biochemical constituents of saliva is of great help in the diagnosis of oral diseases [3, 4] as well as monitoring general health [5]. Saliva contains numerous biochemical markers of pathological processes in periodontal tissue (enzymes, immunoglobulins, growth factors, proteins, lipid peroxidation and oxidative DNA damage markers). [6] In response to periodontal infection, in addition to other biomolecules, numerous enzymes of stromal, epithelial, inflammatory and bacterial cells are released in saliva and gingival fluid. Literature findings suggest that intracellular enzymes responsible for metabolic processes in cells are significantly increased in the saliva of patients with periodontal disease compared to healthy subjects [7-13].

Some studies have found increased activity of aminotransferases [7, 8, 11, 14], and alkaline phosphatase (ALP) in saliva of patients with chronic periodontitis, however, there is little information about their levels in saliva of patients who have aggressive periodontitis.

The aim of this study was to analyze the activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and ALP in saliva of patients with aggressive periodontitis and healthy subjects in relation to clinical parameters of the disease.

MATERIAL AND METHODS

The study included 50 respondents of both genders: 22 with aggressive periodontitis and 28 healthy subjects (control group). The patients with aggressive periodontitis visited the Department of Periodontology, Military Medical Academy in Belgrade in the period 2009-2012. All respondents were healthy with no systemic disease. Pregnant, lactating, menopausal and women receiving estrogen therapy were excluded from the study. During the first clinical examination several parameters: gingival index, bleeding index and probing depth were determined for all respondents. Mixed non-stimulated saliva was collected using special plastic tubes (Salivette®, Sarstedt, Germany

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between 9 a.m. and 11 a.m. Then after saliva samples were centrifuged at 3,000 rpm for 10 minutes.

Enzyme activity was analyzed using kinetic methods and spectrophotometer (Secomam Basic, France) according to the recommendation of the International Federation of Clinical Chemistry (IFCC method). Factory reagents manufactured by Human (Germany) were used. The enzyme activity of AST and ALT was measured in the spectrophotometer at the wavelength of 340 nm, while ALP was determined by measuring the absorbance at the wavelength of 405 nm. Enzyme activity in saliva was expressed in international units per litre (U/L).

In order to perform necessary statistical tests, the statistical software package SPSS (18.0) was used. Numerical variables were described using central tendency and variability measures: mean, median, standard deviation, minimum and maximum value. Numerical data distribution was evaluated using Kolmogorov-Smirnov test. All parameters in the study were non-parametric; therefore Mann-Whitney U-test was used. Correlation between clinical and biochemical parameters was examined using Spearman's correlation coefficient. The level of significance (p) was set at 0.05.

RESULTS

The activity of enzymes ALT and ALP was significantly increased in saliva of patients with aggressive periodontitis compared to the control group (healthy subjects), whereas there was no significant difference between these groups for AST (Table 1).

None of analysed biochemical parameters significantly correlated with clinical signs of disease: gingival index, bleeding index and probing depth (Table 2).

DISCUSSION

Key enzyme markers that appear in saliva during inflammation, connective tissue and bone destruction are important in different biological stages of aggressive periodontitis. Saliva is biological material that can be collected using non-invasive methods and used as additional diagnostic tool for the detection of various enzymes and other biomolecules in the diagnosis of periodontal disease [15, 16]. In our study, the activity of intracellular enzymes AST, ALT and ALP in saliva of patients with aggressive periodontitis was analyzed.

Aminotransferases (AST and ALT) are cytoplasmic enzymes that catalyze amino acid transfer (transamination) during amino acid catabolism in cells. They are important biochemical markers, especially in heart and hepatic diseases. After cell damage these enzymes are released in body fluids where they can be detected as significant biomarkers of acute processes. Many studies have shown that after destruction of periodontal tissue ALT and AST are released in saliva [7, 8, 11, 17] and gingival fluid [18, 19, 20]. In the current study, ALT activity was increased in saliva of patients with aggressive periodontitis, unlike AST. Because pathological processes in periodontal tissue impairs the integrity of cells, increased permeability of plasma membrane results in increased release of enzymes from cytoplasm into saliva. Castro et al. [21] have shown

Table 1. AST, ALT and ALP enzyme activity in saliva of healthy subjects and patients with aggressive periodontitis
Tabela 1. Aktivnost enzima AST, ALT i ALP u pljuvački zdravih i ispitanika obolelih od agresivnog periodontitisa

Enzymes (U/L) Enzimi (U/l)	Healthy subjects Zdravi ispitanici				Patients with aggressive periodontitis Oboleli od agresivnog periodontitisa				p[a]
	X̄±SD	Med	Min	Max	X̄±SD	Med	Min	Max	
AST	20.61±19.60	15	1	91	39.18±39.08	23.5	1	166	0.077
ALT	1.61±0.96	1	1	4	5.86±5.94	4	1	23	0.000*
ALP	15.61±11.37	10	5	52	32.73±35.77	24.5	8	178	0.001*

AST – aspartate aminotransferase; ALT – alanine aminotransferase; ALP – alkaline phosphatase; X – mean value; SD – standard deviation; Med – mediana; Min – minimum value; Max – maximum value; p – significance
* statistically significant difference
[a] Mann-Whitney U-test

AST – aspartat-aminotransferaza; ALT – alanin-aminotransferaza; ALP – alkalna fosfataza; X – aritmetička sredina; SD – standardna devijacija; Med – medijana; Min – najmanja vrednost; Max – najveća vrednost; p – značajnost
* statistički značajna razlika
[a] Man–Vitnijev U-test

Table 2. Correlation between clinical parameters and enzyme activity (AST, ALT, ALP) in saliva of patients with aggressive periodontitis
Tabela 2. Korelacija kliničkih parametara i aktivnosti enzima (AST, ALT, ALP) u pljuvački ispitanika obolelih od agresivnog periodontitisa

Parameters Parametri	AST		ALT		ALP	
	ρ[a]	p	ρ[a]	p	ρ[a]	p
Gingival index Gingivalni indeks	-0.430	0.109	-0.062	0.825	-0.301	0.276
Bleeding index Indeks krvarenja	-0.244	0.381	-0.145	0.607	-0.030	0.916
Probing depth Dubina parodontalnog džepa	0.140	0.619	0.283	0.307	0.145	0.606

[a] Spirman's correlation coefficient
[a] Spirmanov koeficijent korelacije

that the level of AST in gingival fluid becomes increased if periodontal tissue is damaged both in localized and generalized aggressive periodontitis. However, analysis of gingival fluid provides limited information about the condition of certain tissue comparing to analysis of total saliva. Determination of markers in saliva has advantage because it provides information about the condition of periodontal tissue of the whole mouth [15]. Under physiological conditions, AST and ALT appear in saliva in small quantities, and originate from a small number of dead cells [22]. Increased activity of intracellular enzymes in saliva is an indicator of acute process in periodontal tissue. In addition, these enzymes may indicate the degree of periodontal tissue damage due to positive correlation between increased AST and ALT values in saliva and some clinical parameters (gingival index, bleeding index, probing depth) [8, 17]. On the other hand, there is no correlation between ALT levels and plaque index [14]. Our research indicated no correlation between clinical parameters and activities of AST and ALT in saliva in aggressive periodontitis. In contrast to our results, AST activity in gingival fluid increases with increasing values of clinical parameters. Sampling of gingival fluid around individual teeth may indicate different progression of aggressive periodontitis characterized by rapid destruction of periodontal tissue. Our previous research demonstrated reduced activity of intracellular enzymes in saliva in chronic periodontal disease after treatment receiving [7]. Similar results were obtained with matrix metalloproteinases in gingival fluid in localized aggressive periodontitis [23]. The authors believe that intracellular enzymes, and specially ALT, can serve as important biochemical markers of soft periodontal tissue damage in aggressive periodontitis.

Alkaline phosphatase is a membrane enzyme that hydrolyzes different monophosphate esters. Several isoenzymes have been isolated in human tissue: intestinal, placental, placental-like and tissue non-specific isoenzymes. Tissue non-specific ALP isoenzyme has been detected in bone, liver, kidney and oral tissues such as pulp and periodontal tissue. It is produced by many cells such as polymorphonuclear leukocytes, osteoblasts, macrophages, and fibroblasts [24-27]. It has been shown that ALP is released in saliva from polymorphonuclear cells as a result of metabolic changes in inflamed gingiva [12]. The authors believe that this enzyme is an indicator of periodontal cell damage in gingivitis. Literature data suggests correlation between ALP activity in gingival fluid and periodontal disease [21, 28]. Most studies have been conducted in patients with chronic periodontitis [7, 10, 13, 14]. In the current study, increased ALP activity in non stimulated saliva was demonstrated in aggressive periodontitis but not in healthy subjects. Since biological function of ALP is in bone mineralization [29], its increased presence in saliva of patients with aggressive periodontitis is the result of rapid alveolar bone destruction and cell membrane damage of osteoblasts and fibroblasts. Our research did not find significant correlation between ALP activity and clinical parameters, which is consistent with other studies [14]. However, other studies have also shown positive correlation between ALP activity and periodontal pocket depth [10] and gingival index [7].

CONCLUSION

Correlation between levels of intracellular enzymes AST, ALT and ALP in saliva and pathological changes in aggressive periodontitis reflects changes at molecular level in periodontal tissue. Therefore, these enzymes may be potential biochemical markers for the detection and progression of this disease.

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Analiza unutarćelijskih enzima u pljuvački osoba obolelih od agresivnog periodontitisa

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KRATAK SADRŽAJ

Uvod Agresivni periodontitis se odlikuje veoma brzim propadanjem parodontalnog tkiva i češće se javlja kod mladih ljudi. Analiza biohemijskih markera u pljuvački je vrlo značajna za utvrđivanje stepena oštećenja parodontalnog tkiva. Cilj studije je bio analiza unutarćelijskih enzima – aspartat-aminotransferaze (AST), alanin-aminotransferaze (ALT) i alkalne fosfataze (ALP) – u pljuvački osoba obolelih od agresivnog periodontitisa.

Materijal i metode rada U studiju su uključena 22 pacijenta s agresivnim peridontitisom, dok je kontrolnu grupu činilo 28 zdravih ispitanika. Aktivnost enzima je analizirana u mešovitoj nestimulisanoj pljuvački kinetičkim metodama na spektrofotometru, a izražena je u internacionalnim jedinicama po litru (U/l).

Rezultati Aktivnost enzima ALT i ALP bila je statistički značajno povećana u pljuvački ispitanika obolelih od agresivnog periodontitisa u odnosu na grupu zdravih ispitanika, dok se aktivnost AST nije statistički značajno razlikovala. Takođe, nije postojala korelacija između aktivnosti unutarćelijskih enzima u pljuvački i kliničkih parametara (gingivalnog indeksa, indeksa krvarenja, dubine parodontalnog džepa).

Zaključak Dobijeni rezultati pokazuju da su enzimi AST, ALT i ALP u pljuvački potencijalni biohemijski markeri za pomoć u dijagnostikovanju agresivnog periodontitisa.

Ključne reči: agresivni periodontitis; ALP; ALT; AST; unutarćelijski enzimi; pljuvačka

UVOD

Agresivni periodontitis je oboljenje praćeno intenzivnim propadanjem potpornog aparata zuba. Dijagnoza se zasniva na kliničkim parametrima (plak-indeks, gingivalni indeks, dubina parodontalnog džepa, indeks krvarenja, nivo pripojnog epitela) i radiografskim parametrima (stepen gubitka alveolarne kosti) [1, 2]. Ova klinička merenja su korisna za dijagnostikovanje periodontitisa, ali pružaju vrlo ograničene informacije u vezi sa supkliničkim oblicima oboljenja, prognozom ove bolesti i procenom efekata primenjene terapije. Analiza biohemijskog sastava pljuvačke može biti značajna kao dopunski dijagnostički test.

Pljuvačka je sekret složenog sastava i specifičnih uloga. Analiza biohemijskih sastojaka pljuvačke je od velike pomoći u dijagnostici, kako kod oboljenja usne duplje [3, 4], tako i kod praćenja opšteg zdravstvenog stanja organizma [5]. U pljuvački se mogu dokazati brojni biohemijski markeri patološkog procesa u parodoncijumu: enzimi, imunoglobulini, faktori rasta, proteini, markeri lipidne peroksidacije i oksidativnog oštećenja DNK [6]. Kao odgovor organizma na parodontalnu infekciju, pored ostalih biomolekula, oslobađaju se mnogi enzimi u pljuvačku i gingivalnu tečnost iz stromalnih, epitelnih, inflamatornih ili bakterijskih ćelija. Podaci iz literature pokazuju da je koncentracija unutarćelijskih enzima, koji su odgovorni za odigravanje metaboličkih procesa u ćelijama, znatno povećana u pljuvački osoba obolelih od parodontopatije u odnosu na zdrave ljude [7-13].

Neke studije su opisale da je aktivnost aminotransferaza [7, 8, 11, 14] i alkalne fosfataze (ALP) povećana u pljuvački osoba obolelih od hronične parodontopatije, ali vrlo malo podataka ima o njihovom nivou u pljuvački obolelih od agresivnog periodontitisa.

Cilj studije je bio da se analizira aktivnost aspartat-aminotransferaze (AST), alanin-aminotransferaze (ALT) i ALP u pljuvački osoba obolelih od agresivnog periodontitisa u odnosu na kliničke parametre ovog oboljenja i grupu zdravih ispitanika.

MATERIJAL I METODE RADA

U studiju je uključeno 50 ispitanika oba pola: 22 osobe s agresivnim periodontalnim oboljenjem i 28 zdravih ispitanika, koji su činili kontrolnu grupu. Pacijenti s oralnim problemima posetili su Kliniku za parodontologiju Vojnomedicinske akademije u Beogradu u periodu 2009–2012. godine. Sve osobe su bile dobrog opšteg zdravlja i bez sistemskih oboljenja. U studiju nisu bile uključene trudnice i dojilje, žene u menopauzi i one na estrogenoj terapiji. Tokom prve posete ispitanicima je urađen klinički pregled koji je obuhvatio određivanje vrednosti gingivalnog i indeksa krvarenja i dubine parodontalnog džepa.

Mešovita nestimulisana pljuvačka je sakupljana pomoću specijalnih plastičnih epruveta (*Salivette®*, Zaršted, Nemačka) između devet i jedanaest časova ujutro. Zatim su uzorci pljuvačke centrifugirani na 3.000 obrtaja deset minuta.

Aktivnost enzima je analizirana kinetičkim metodama na spektrofotometru (*Secomam Basic*, Francuska) prema preporukama Međunarodne federacije za kliničku hemiju (metoda IFCC). Pri tome su korišćeni fabrički reagensi proizvođača *Human* (Nemačka). Aktivnost AST i ALT merena je na spektrofotometru na talasnoj dužini od 340 nm, dok je određivanje aktivnosti ALP vršeno merenjem apsorbance na talasnoj dužini od 405 nm. Aktivnost enzima u pljuvački izražena je u internacionalnim jedinicama po litru (U/l).

Radi izvođenja neophodnih statističkih testova, korišćen je statistički programski paket SPSS 18.0. Posmatrane numeričke varijable opisane su klasičnim merama centralne tendencije i merama varijabiliteta: aritmetičkom sredinom, medijanom, standardnom devijacijom i najmanjom i najvećom vrednošću. Izbor testova za analizu numeričkih obeležja posmatranja zavisio je od prirode njihove raspodele, koja je ispitivana Kolmogorov–Smirnovljevim (*Kolmogorov–Smirnov*) testom. Svi parametri u našoj studiji bili su neparametrijski, zbog čega je

za utvrđivanje razlike u njihovim vrednostima između posmatranih grupa korišćen Man–Vitnijev (*Mann–Whitney*) U-test. Povezanost kliničkih i biohemijskih parametara ispitivana je Spirmanovim (*Spearman*) koeficijentom korelacije. Granična vrednost za prihvatanje hipoteze o postojanju razlike između testiranih grupa u analiziranim varijablama postavljena je na $p<0,05$.

REZULTATI

Aktivnost enzima ALT i ALP bila je statistički značajno povećana u pljuvački ispitanika obolelih od agresivnog periodontitisa u odnosu na kontrolnu grupu, dok se aktivnosti AST nije statistički značajno razlikovala (Tabela 1).

Nijedan posmatrani biohemijski parametar nije statistički značajno korelirao s analiziranim kliničkim znacima bolesti: gingivalnim indeksom, indeksom krvarenja i dubinom parodontalnog džepa (Tabela 2).

DISKUSIJA

Definisanje ključnih enzimskih markera koji se pojavljuju u pljuvački tokom zapaljenja, vezivno-tkivne i koštane destrukcije važni su za različite biološke faze agresivnog periodontitisa. Pljuvačka je biološki materijal koji je lako dostupan neinvazivnim metodama i u kojem se dokazuju različiti enzimi i drugi biomolekuli kao pomoć u dijagnostikovanju parodontalnih oboljenja [15, 16]. U našem istraživanju je analizirana aktivnost unutarćelijskih enzima AST, ALT i ALP u pljuvački osoba s agresivnim periodontitisom.

Aminotransferaze (AST i ALT) su citoplazmatski enzimi koji katalizuju prenos aminogrupe (transaminacija) tokom procesa katabolizma aminokiselina u ćelijama. Oni su značajni biohemijski markeri, posebno kod oboljenja srčanog mišića i hepatičnog tkiva. Nakon oštećenja ćelija oslobađaju se u telesne tečnosti, gde se dokazuju kao značajni biomarkeri akutnih procesa. Mnoge studije su pokazale da tokom propadanja parodontalnog tkiva dolazi do oslobađanja AST i ALT u pljuvačku [7, 8, 11, 17] i gingivalnu tečnost [18, 19, 20]. U našoj studiji aktivnost ALT je bila povećana u pljuvački ispitanika s agresivnim peridontitisom, za razliku od aktivnosti AST. Usled patološkog procesa u parodontalnom tkivu, narušava se integritet ćelija, povećava propustljivost plazma-membrana, pa se enzimi pojačano oslobađaju iz citoplazme ćelija u pljuvačku. Kastro (*Castro*) i saradnici [21] su dokazali da je nivo AST u gingivalnoj tečnosti povećan kod većih oštećenja parodontalnog tkiva, kako kod lokalizovanog, tako i kod generalizovanog oblika agresivnog periodontitisa. Međutim, analiza gingivalne tečnosti daje ograničene informacije o stanju tkiva pojedinih zuba u odnosu na analizu ukupne pljuvačke. Određivanje markera u pljuvački ima neke prednosti, jer se dobija objedinjena informacija o stanju parodontalnih tkiva svih zuba [15]. U fiziološkim uslovima AST i ALT se pojavljuju u pljuvački u vrlo malim količinama, a potiču od malog broja izumrlih ćelija [22]. Povećana aktivnost unutarćelijskih enzima u pljuvački je pokazatelj akutnog procesa u parodontalnim tkivima. Pored toga, ovi enzimi mogu biti pokazatelji stepena oštećenja parodontalnih tkiva, jer je ustanovljena pozitivna korelacija između povećane aktivnosti AST i ALT u pljuvački i vrednosti nekih kliničkih parametara (gingivalni indeks, indeks krvarenja, dubina parodontalnog džepa) [8, 17], dok korelacije između nivoa ALT i plak-indeksa nema [14]. Naše istraživanje je pokazalo da kod agresivnog periodontitisa ne postoji korelacija između kliničkih parametara i aktivnosti AST i ALT u pljuvački. Nasuprot našim rezultatima, aktivnost AST u gingivalnoj tečnosti se povećava s porastom vrednosti kliničkih parametara. Uzimanje uzoraka gingivalne tečnosti oko pojedinih zuba može da ukaže na različitu progresiju agresivnog periodontitisa, koja se odlikuje vrlo brzim propadanjem parodontalnog tkiva. U našim ranijim istraživanjima je dokazana smanjena aktivnost unutarćelijskih enzima u pljuvački osoba s hroničnim parodontalnim oboljenjem nakon primenjene terapije [7]. Slični rezultati su dobijeni i sa matriksnim metaloproteinazama u gingivalnoj tečnosti ispitanika s lokalizovanim agresivnim periodontitisom [23]. Smatramo da unutarćelijski enzimi – a posebno ALT – mogu poslužiti kao značajni biohemijski markeri oštećenja mekog parodontalnog tkiva tokom brzog oštećenja tkiva usled agresivnog periodontitisa.

ALP je membranski enzim koji hidrolizuje različite monofosfatne estre. U ljudskim tkivima je izolovano nekoliko izoenzima: intestinalni, placentni, izoenzim sličan placentnom i tkivni nespecifični izoenzim. Tkivni nespecifični izoenzim ALP je dokazan u kostima, jetri, bubrezima i oralnim tkivima, kao što su pulpa i periodontalna tkiva. Proizvode ga mnoge ćelije, poput polimorfonuklearnih leukocita, osteoblasta, makrofaga i fibroblasta [24-27]. Dokazano je da se kao posledica metaboličkih promena u zapaljenoj gingivi ALP oslobađa iz polimorfonuklearnih ćelija u pljuvačku [12]. Autori smatraju da je kod gingivitisa ovaj enzim pokazatelj oštećenja ćelija mekih tkiva parodoncijuma. U literaturi postoje podaci o korelaciji aktivnosti ALP u gingivalnoj tečnosti i periodontalnih oboljenja [21, 28]. Uglavnom su istraživanja rađena kod pacijenata s hroničnim periodontitisom [7, 10, 13, 14]. U našem istraživanju je kod ispitanika obolelih od agresivnog periodontitisa ustanovljena povećana aktivnost ALP u nestimulisanoj pljuvački u odnosu na zdrave ispitanike. S obzirom na to da je biološka funkcija ALP u mineralizaciji kosti [29], njegova povećana koncentracija u pljuvački osoba s agresivnim periodontitisom posledica je brzog propadanja alveolarne kosti i oštećenja membrana ćelija osteoblasta i fibroblasta. U našem istraživanju nije dokazana povezanost aktivnosti ALP i vrednosti kliničkih parametara, što je u saglasnosti s nalazima istraživanja hroničnog periodontitisa [14], dok su druge studije utvrdile pozitivnu korelaciju aktivnosti ALP i dubine parodontalnog džepa [10] i gingivalnog indeksa [7].

ZAKLJUČAK

Povezanost nivoa unutarćelijskih enzima AST, ALT i ALP u pljuvački i patoloških promena u agresivnom periodontitisu odražava promene na molekularnom nivou u parodontalnom tkivu. Zato ovi enzmi mogu biti potencijalni biohemijski markeri za otkrivanje i progresiju ovog oboljenja.