

Effect of Dental Prophylaxis on the Salivary Cortisol Levels and Alpha-Amylase Activity in Children

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The purpose of this study was to assess the pattern of changes in salivary cortisol, alpha-amylase and total protein in children undergoing dental prophylaxis, moreover, to investigate the effect of prior preparation to the intervention. 38 (9-10 year-old) children were equally divided into test and control group. Unstimulated whole saliva was collected before and after professional dental prophylaxis. Test group participated in a dentistry-related demonstration. Changes in salivary alpha-amylase, cortisol and total protein level were assessed. The test and the control groups showed divergent salivary cortisol level variations, at the test group the demonstration provoked a primary increase, which was even more enhanced at the end of prophylaxis, while the control group presented a reverse, decreasing tendency. Alpha-amylase showed similar baseline activities at the two groups, with slightly different, but non-significant decrease as test effect. The statistically significant increase of salivary cortisol and the correlating in-test changes of cortisol and amylase at the test group in opposite to controls, strongly suggests that the presentation was the actual stressor in this study.

Keywords: Salivary biomarkers of stress, salivary cortisol, salivary alpha-amylase, children, dental prophylaxis

Anxiety and stress are frequently related to dental treatment even in children who did not previously go through such a procedure. Dental fear and anxiety (DFA) is regarded as a form of psychological stress, thus, has a physiological impact on the body, similar to those triggered by physical challenges [1]. Two primary systems are particularly involved in setting on the stress response, the hypothalamus-pituitary-adrenocortical axis (HPA) and the sympatho-adrenomedullary system (SAM) [2].

Many studies have shown that various kinds of psychological stress activate the HPA, which causes an increase in cortisol secretion in the adrenal cortex. It has been also suggested that salivary cortisol concentration may be related to the level of dental anxiety. Moreover, salivary cortisol concentrations are closely correlated to serum cortisol levels [3-6].

Alpha-amylase is one of the major salivary enzymes in humans, secreted from the salivary glands in response to sympathetic stimuli [7-9]. Secretion of salivary alpha-amylase (SAA) is regulated by norepinephrine via the sympatho-adrenomedullary system and its activity is reported to increase during distress and decrease during comfort [2, 10, 11]. It was reported that there was a good association between the concentration of salivary amylase and blood levels of catecholamines [10]. Currently, it is considered that the measurement of this salivary enzyme is a reliable biomarker of stress response in clinical settings as well as being a useful tool for evaluating the SAM system [2, 12-14].

Thus, salivary cortisol and alpha-amylase, also known as salivary biomarkers of stress, reliably reflect the HPA and SAM systems activity, respectively. In addition, the collection of saliva is a non-invasive method and it therefore has a major advantage in stress research. It represents a more practical assessment tool than blood collection due to elimination of the hyperstress component of the latter [15, 16].

The purpose of this study was to assess the pattern of changes in salivary biomarkers (salivary cortisol, alpha-amylase and total protein) in children undergoing a minor dental procedure (dental prophylaxis). In addition, we also investigated the effect of prior preparation to the intervention. Together with the personal history, the oral hygiene index and the decayed, missing and filled teeth (DMFT) index has been assessed and correlated to both cortisol levels and amylase activity.

Experimental part

A total of 38 healthy children consisting of 20 females and 18 males, between the ages of 9 and 10 years were included. The aim of the study and the procedures involved were explained to the subjects and their parents, after which the latter gave written consent. The Ethical Research Committee of the University of Medicine and Pharmacy of Tîrgu-Mure^o approved the study protocol.

The subjects were equally divided into a test and control group, based on age and gender. No further randomization

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protocol was applied. A short demonstration about the necessity of oral hygiene and dental procedures was presented to the test group before the first saliva collection and the dental prophylaxis. Saliva samples were collected before the demonstration to measure the baseline (pre-demonstration) levels of cortisol, total protein, and also amylase activity. Professional dental prophylaxis was performed on both groups and whole saliva samples were collected before and after (pre- and post-intervention) at the test group, and controls. Cortisol level (Δ cortisol) and amylase activity (Δ amylase) variations were defined as [(post-intervention value) - (pre-intervention value)], also referred as in-test variations of the two parameters.

Experimental sessions took place in the morning (between 9 and 11 a.m.) to minimize the effects of diurnal changes. Salivary alpha-amylase activity has a pronounced and distinct diurnal rhythm with a strong drop in activity in the first hour after awakening and a steady increase towards the evening. It is independent of the diurnal rhythm of free cortisol, but it is influenced by age [3, 17].

Subjects were instructed on the method of saliva collection, which lasted for 5 min in both sessions. Immediately after collection, the two saliva samples from each subject were stored at -20°C until the samples were analyzed.

Quantitative determination of salivary cortisol (mg/mL) was performed by a saliva specific ELISA method with the Salivary Cortisol Enzyme Immunoassay Kit, a product of Salimetrics-USA. The Kinetic Enzyme Assay Kit of Salimetrics-USA was used for the analysis of salivary alpha-amylase (expressed as U/mL). Meanwhile the salivary total protein level was determined with the Bradford micro-method and expressed as g/L. Since the distribution of cortisol concentrations and amylase activity were non-gaussian, non-parametric tests were applied in statistical evaluation: the Mann-Whitney U test was used in between-group comparison of parameters, the Spearman rank was applied for correlation analysis, while the Wilcoxon matched-pairs test served for intra-group comparisons. The threshold of statistical significance was set to $p \leq 0.05$.

Results and discussions

A wide range-variability of salivary amylase activity and cortisol levels was registered. The changes in normalized salivary cortisol, α -amylase are shown in (fig.1a.-b.) and (fig.2a.-b.). Concentration of salivary total protein initially was significantly different between the two groups, and showed only slight in-test variations. In order to overcome variability due to dilution/concentration of saliva, cortisol levels and amylase activities were normalized to the concentration of total proteins.

Salivary cortisol

The initial levels of salivary cortisol were somewhat higher at the test group than at the control group (0.159 vs. 0.133, $p=0.07$) (table 1 and table 2). The post-demonstration cortisol levels at the test group were significantly higher than baselines of controls (0.192 vs. 0.133, $p<0.001$) and tended to a significant increase at the end of the prophylaxis. Concerning cortisol level variations, the post-intervention values of test group were significantly higher than at baseline and post-demonstration (0.211 vs. 0.191 and 0.159, $p=0.006$ and $p=0.015$) (fig. 1a., table 1). Contrarily, a decrease with tendency to significance was observed at controls (0.133 vs. 0.118, $p=0.058$) (fig.1b., table2). 63% patients of the test group, but only 21% of controls showed an increase in normalized salivary cortisol levels after prophylaxis.

Amylase activity

Although amylase activity at baseline tended to a statistical difference in favour of controls, the normalized values were similar. At the test group, the demonstration provoked a slight, but non-significant increase in activity (2.41 vs. 2.25, $p=0.16$), followed by a drop from the beginning to the end of the prophylaxis (2.41 vs. 1.99, $p=0.63$), a trend also observed at the control group (2.61 vs. 2.16, $p=0.42$) - all variations being without significance (table 1 and 2, fig. 2a.-b.).

The post-intervention values were comparable between the test and control group. An in-test raise of amylase activities were detected at 58% of the test group members and 42% of the controls (fig. 2a.-b.).

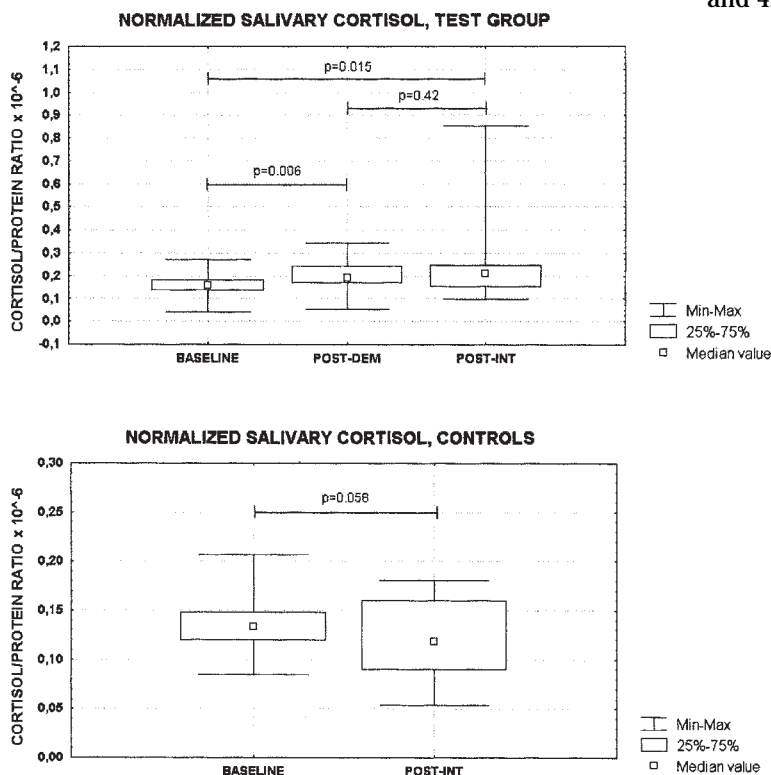


Fig. 1a.-b. Variations of cortisol levels in the test and control groups. Normalized cortisol activity shown as ratio ($\mu\text{g/g}$, 10^{-6}), median (25-75% percentiles, minimum-maximum). p values calculated according to the Wilcoxon matched-pairs test.

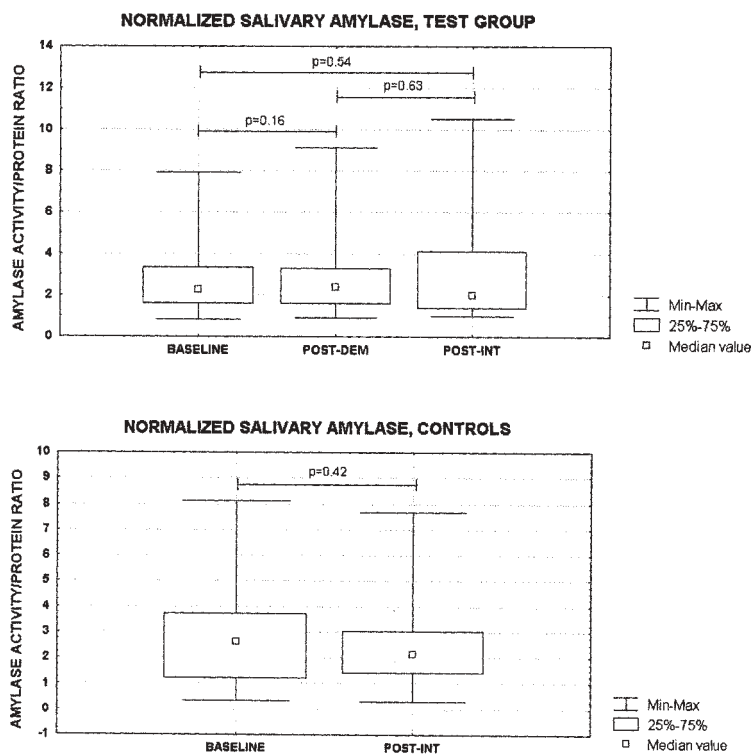


Fig. 2a.-b. Variations of amylase activity in the test and control groups. Normalized amylase activity shown as (U/mg), median (25-75% percentiles, minimum-maximum). p values calculated according to the Wilcoxon matched-pairs test

Median±Perc.range			
Parameters	Test group (n=19)	Control group (n=19)	p value
Baseline cortisol (µg/L)	8.36 (7.25 – 10.35)	10.70 (9.51 – 12.21)	0.016
Baseline cortisol, normalized (µg/g, 10 ⁻⁶)	0.159 (0.14 – 0.18)	0,133 (0.12 – 0.15)	0.065
Baseline amylase (U/mL)	122.78 (88.98 – 187.67)	194.99 (103.32 – 311.10)	0.074
Baseline amylase, normalized (U/mg)	2.25 (1.55 – 3.37)	2.61 (1.20 – 3.69)	0.82
Baseline total protein (g/L)	55.75 (50.71 – 62.44)	79.73 (73.2 – 86.27)	<0.001
Pre-intervention cortisol (µg/L)	11.73 (10-12.51)	-	
Pre-intervention cortisol, normalized (µg/g, 10 ⁻⁶)	0.192 (0.168-0.244)	-	
Pre-intervention amylase (U/mL)	134.15 (82.49-204.51)	-	
Pre-intervention amylase, normalized (U/mg)	2.41 (1.58-3.30)	-	
Pre-intervention total protein (g/L)	61 (34.31-66.23)	-	

Table 1
BASELINE AND PRE-INTERVENTION PARAMETERS IN THE STUDY GROUPS

Correlations of salivary cortisol and amylase

The influence of the DMFT index, an important covariate of the stress markers, has been correlated to the salivary cortisol levels and amylase activity, and also their variations. No important correlation with the aforementioned parameters has been assessed.

Furthermore, we could not detect any significant correlation between the oral hygiene index and initial, final levels or variations of cortisol and amylase.

In the test group, there was an association between cortisol and amylase variations (positive correlation between D cortisol and D amylase, $r=0.50$, $p=0.029$).

Many studies have shown that psychological stressors, such as academic examination period [15,18], psychosocial stress [19-21], suspense video viewing [2, 22] and dental procedures [6, 23-25] can induce significant increases in salivary cortisol and amylase levels. Takai et

al. [2] have clearly shown that the salivary amylase level increased more significantly and reacted more rapidly than cortisol by psychological stressor, suggesting that it is a better index of stress.

Miller et al. demonstrated that salivary cortisol levels in dental treatment are the highest in patients undergoing tooth extraction compared to other procedures such as prophylaxis, restorative treatments and examination. They investigated the adrenal stress response to various dental treatments in healthy adults and reported that cortisol levels measured at the start of a dental procedure decreased in patients undergoing non-invasive dental procedures, such as routine examinations. Conversely, cortisol levels at the end of tooth extractions were elevated compared with baseline cortisol [1, 11, 26]. In addition, recent studies have reported significantly higher salivary α -amylase levels in children before dental treatment compared to afterward,

<i>Median ± Percentile range</i>			
Parameters	Test group (n=19)	Control group (n=19)	p value
Post test cortisol	11.06 (9.52 – 12.41)	9.90 (7.11 – 11.52)	0.049
Normalized post test cortisol	0.211 (0.151 – 0.246)	0.118 (0.084 – 0.160)	<0.001
Post test amylase	127.59 (72.48-191.55)	164.32 (100.86 – 257.15)	0.258
Normalized post test amylase	1.99 (1.32 – 4.11)	2.15 (1.38 – 3.02)	0.840
Post test total protein	60.12 (44.87 – 69.71)	78.86 (71.89 – 87.14)	<0.001

Table 2
POST-TEST COMPARISON OF PARAMETERS
BETWEEN GROUPS

which indicates that anticipation of treatment may cause an exacerbated salivary α -amylase response [11, 23, 27].

In our study, dental prophylaxis was supposed to represent the stressor, being applied on both test and control groups, respectively. Oral prophylaxis is a noninvasive, relatively easy and ethical procedure, which is considered to be painless even in children with severe dental caries. Therefore it was assumed that alteration in salivary cortisol and amylase levels would be due to stress and not pain [28]. In addition, the members of the test group were informed about dental hygiene as well as different dental treatments before the prophylactic intervention took place.

In our study, the step-by-step increase of cortisol levels in the test group from the beginning to the end-point by our opinion points to the suspicion that the presentation prior to the intervention had a stressing effect. This was also supported by the fact that in the meantime, the control group displayed a tendency for statistically significant decrease in the cortisol level.

This presumption was also enforced by the observations made on the variation of α -amylase within and amongst the two groups. Although the baseline levels were similar in the two groups, the test group presented a slight initial increase of α -amylase after the demonstration, followed by a non-significant decrease at post-intervention, whilst the control group also showed a drop in the level of this enzyme. This is similar to Takai et al.'s observation, where it was suggested that the enzyme was a soothing or relaxation index and that the amylase measurement would be a powerful tool for psychological research [2]. Amylase activity, as a quickly reacting stress marker, is reasonably enhanced even in the pre-intervention period.

Kambalimath et al. have revealed no significant differences in the mean salivary cortisol levels between caries and cariesfree groups before subjecting the children to dental prophylaxis. Although a significant increase in post-fluoride treatment salivary cortisol levels was reported at the first appointment irrespective of the caries activity in children, this trend was not evident in the second appointment in both groups. These results suggest that the stressors selected for the study were indeed appropriate, whilst also suggesting that children are able to adapt to the stressors by the second appointment [28]. Based on salivary alpha-amylase activity changes, Arhakis et al. also reported that dental treatment involving local anesthesia in naive children appeared to be less stressful after 3 sessions [24].

The inverse relationship between the control and test group in our study may be also due to a complex interplay of factors which in turn affects the behavior of children during treatment. Overcoming fear may require more

mental activity by the brain's cortex, which may subsequently cause more stimulation of autonomic nervous system. This will result in higher alpha-amylase levels in children with a more positive behavior [14].

Conclusions

The step-by step increase of salivary cortisol levels during the demonstration and prophylaxis in the test group was in contrast with the slight initial increase and than fall of amylase activity. This highlights the fact that the prophylactic intervention itself was not stressful for the subjects, but moreover, had a soothing effect. Above all, the statistically significant increase of the salivary cortisol in the test group strongly suggests that the presentation was in fact the real stressor in this study. Due to the complex interplay of factors affecting child behavior during treatment, further studies on larger samples are required to be able to draw more appropriate conclusions.

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References

- SADI, H., FINKELMAN, M., ROSENBERG, M., Salivary cortisol, salivary alpha amylase and the Dental Anxiety Scale. *Anesth Prog*, nr. 60, 2013, p: 46-53.
- TAKAI, N., YAMAGUCHI, M., ARAGAKI, T., ETO, K., UCHIHASHI, K., NISHIKAWA, Y., Effect of psychological stress on the salivary cortisol and amylase levels in healthy young adults. *Archives of Oral Biology*, nr. 49, 2004, p: 963-968.
- KIRSCHBAUM, C., HELLHAMMER, DH., Salivary cortisol in psychoneuroendocrine research: recent developments and applications. *Psychoneuroendocrinology*, nr. 19, 1994, p: 313-333.
- SAPOLSKY, R., KREY, LC., MCEWEN, BS., The neuroendocrinology of stress and aging: the glucocorticoid cascade hypothesis. *Endocr Rev*, nr. 7, 1986, p: 284-301.
- AARDAL, E., HOLM, AC., Cortisol in saliva-reference ranges and relation to cortisol in serum. *Eur J Clin Chem Clin Biochem*, 12, nr. 33, 1995, p: 927-932.
- BENJAMINS, C., ASSHCEMAN, H., SCHUURS, AH., Increased salivary cortisol in severe dental anxiety. *Psychophysiology*, nr. 29, 1993, p: 302-305.
- GALLACHER, DV., PETERSEN, OH., Stimulus-secretion coupling in mammalian salivary glands. *Int Rev Physiol*, nr. 28, 1983, p: 1-52.
- DESHPANDE, RR., PANVALKAR, PS., KULKARNI, AA., GADKARI, TV., Age-related Changes of the Human Salivary Secretory Total Protein Complex and Trace Elements in Children between the Age Group of 3-16 years. *J Biomed Sci and Res*, 1, nr. 3, 2011, p: 362-367.

- 9.SIVAKUMAR, T., HAND, AR., MEDNIEKS, M., Secretory proteins in the saliva of children. *Journal of Oral Science*, 4, nr. 51, 2009, p: 573-580.
- 10.CHATTERTON, RT., VOGELSONG, KM., LU, YC., ELLMAN, AB., HUDGENS, GA., Salivary alpha-amylase as a measure of endogenous adrenergic activity. *Clin Physiol*, 4, nr.16, 1996, p: 433-448.
- 11.KARIBE, H., AOYAGI-NAKA, K., KODA, A., Maternal anxiety and child fear during dental procedures: a preliminary study. *Journal of Dentistry for Children*, 2, nr. 81, 2014, p: 72-78.
- 12.WALSH, NP, BLANNIN, AK., CLARK, AM., COOK, L., ROBSON, PJ., GLEESON, M., The effects of high-intensity intermittent exercise on saliva IgA, total protein and alpha-amylase. *J Sports Sci*, nr. 17, 1999, p: 129-134.
- 13.KARIBE, H., AOYAGI, K., KODA, A., KAWAKAMI, T., Characteristics of the salivary alpha-amylase level in resting sublingual saliva as an index of psychological stress. *Stress Health*, nr 27, 2011, p: 282-288.
- 14.NOORANI, H., JOSHI, HV., SHIVAPRAKASH, PK., Salivary Alpha Amylase as a Noninvasive Biomarker for Dental Fear and Its Correlation with Behavior of Children during Dental Treatment. *International Journal of Paediatric Dentistry*, 1, nr. 7, 2014, p: 19-23.
- 15.NG, V., Stress and Salivary Biomarkers Among Dental Undergraduates - a Longitudinal Study. National University of Singapore, Singapore, 2006, p: 17-24.
- 16.NATER, UM., ROHLEDER, N., Salivary alpha-amylase as a non-invasive biomarker for the sympathetic nervous system: Current state of research. *Psychoneuroendocrinology*, 4, nr. 34, 2009, p: 486-496.
- 17.NATER, UM., ROHLENDER, N., SCHLOTZE, W., EHLERT, U., KIRSCHBAUM, C., Determinants of the diurnal course of salivary alpha-amylase. *Psychoneuroendocrinology*, 4, nr. 32, 2007, p: 392-401.
- 18.MATOS-GOMES, N., KATSURAYAMA, M., MAKIMOTO, FH., SANTANA, LL., PAREDES-GARCIA, E., BECKER, MA., DOS-SANTOS, MC., Psychological stress and its influence on salivary flow rate, total protein concentration and IgA, IgG and IgM titers. *Neuroimmunomodulation*. 6, nr.17, 2010, p: 396-404.
- 19.ROHLEDER, N., NATER, UM., WOLF, JM., EHLERT, U., KIRSCHBAUM, C., Psychosocial stress-induced activation of salivary alpha-amylase: an indicator of sympathetic activity? *Ann. N. Y. Acad. Sci.*, nr. 1032, 2004, p: 258-263.
- 20.NATER, UM., ROHLEDER, N., GAAB, J., BERGER, S., JUD, A., KIRSCHBAUM, C., EHLERT, U., Human salivary alpha-amylase reactivity in a psychosocial stress paradigm. *Int. J. Psychophysiol*, nr. 55, 2005, p: 333-342.
- 21.RASHKOVA, M., KALCHEV, P., EMILOVA, R., RIBAGIN, L., DOGANOVA-TZ., STOEVA, I., Cortisol in saliva - a marker for increased anxiety in children. *Journal of IMAB*, 4, nr. 16, 2010, p: 67-69.
- 22.KODA, A., KARIBE, H., Subjective ratings and autonomic responses to dental video stimulation in children and their mothers. *Pediatric Dental Journal*, nr. 23, 2013, p: 79-85.
- 23.FURLAN, NF., GAVIAO, M., BARBOSA, TS., NICOLAU, J., CASTELO, PM., Salivary cortisol, alpha-amylase and heart rate variation in response to dental treatment in children. *Clin Pediatr Dent*, 1, nr. 37, 2012, p: 83-87.
- 24.ARHAKIS, A., MENEXES, G., COOLIDGE, T., KALFAS, S., Heart rate, salivary alpha-amylase activity and cooperative behavior in previously naive children receiving dental local anesthesia. *Pediatr Dent*, 7, nr. 34, 2012, p: 225-230.
- 25.ALFAHAD, DW., AL-HADITHY, EMR., Dental anxiety and its relation to serum cortisol level before dental surgical treatment. *Anb Med J*, 1, nr. 10, 2012, p: 35-40.
- 26.MILLER, C., DEMBO, J., FALACE, D., KAPLAN, A., Salivary cortisol response to dental treatment of varying stress. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*, nr. 79, 1995, p: 436-441.
- 27.DOS SANTOS, MJ., BERNABEE, D., NAKAMUNE, AC., PERRI, SH., DE AGUIAR, SM., DE OLIVEIRA, SH., Salivary alpha amylase and cortisol levels in children with global developmental delay and their relation with the expectation of dental care and behavior during the intervention. *Res Dev Disabil*, nr. 33, 2012, p: 499-505.
- 28.KAMBALIMATH, HV., DITIX, UB., THYAGI, PS., Salivary cortisol response to psychological stress in children with early childhood caries. *Indian J Dent Res*, 2, nr. 21, 2010, p: 231-237

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