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The activity of salivary aldehyde dehydrogenase during the menstrual cycle and pregnancy

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ABSTRACT

Objective: The aim of the present study was to describe the changes in the activity of ALDH3A1 in saliva in relation to the menstrual cycle and pregnancy. We also measured major salivary antioxidants, salivary peroxidase (SPO) activity and uric acid (UA) concentration.

Design: Fasting saliva samples were collected from 63 women with uncomplicated pregnancies and from 39 healthy women of reproductive age, but not pregnant. Saliva samples were also collected from 10 healthy women with regular menstrual cycles in the early follicular, the mid-cycle and the mid-luteal phase during one menstrual cycle. SPO and ALDH3A1 activity was determined fluorimetrically, whereas UA concentration photometrically.

Results: The ALDH3A1 did not vary significantly among phases of menstrual cycle. However, the enzyme activity decreased with the length of pregnancy and in the third trimester is significantly lower than that in the saliva of non-pregnant women.

Conclusions: Lower concentration of UA and in the third trimester the activity of ALDH3A1 in saliva of pregnant women could be a risk factor of, e.g. oral pathologies.

1. Introduction

The most important salivary antioxidant defence system consists of enzymes, such as salivary peroxidase and some molecules, such as uric acid.[1] Another enzyme related indirectly to the protection against effects of oxidative stress is aldehyde dehydrogenase (ALDH3A1).[2] Salivary ALDH3A1 is very vulnerable to oxidation and reversible inactivation, even by the oxygen in the air. The oxidation may be prevented by glutathione (GSH) and reversed by dithiothreitol (DTT) addition in vitro.[3]

The composition of saliva changes as a result of, for example, systemic inflammatory response.[4,5] Since salivary malondialdehyde (MDA) levels reflect circulating MDA levels well,[6] the examination of saliva may correspond to the organism's multi-systemic antioxidant capacity.

Pregnancy is a condition which exhibits increased susceptibility to oxidative stress defined as a disturbance in the pro-oxidant and antioxidant balance. Moreover, oxidative stress is believed to be a main reason of pregnancy complications, such as preeclampsia, gestational diabetes,[7] hypertension.[8] Reactive oxygen species (ROS) also affect a gamut of physiological functions in female menstrual cycle i.e., oocyte maturation, ovarian steroidogenesis, ovulation and luteolysis.[9]

Changes in the saliva composition during pregnancy and the menstrual cycle can be related to the variation of ROS production,[9] as well as to the interaction of estrogens with the oestrogen receptor in the salivary glands[10] and with the

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oestrogen response element present in the upstream regions of some genes.[4]

The aim of the present study was to examine the influence of the menstrual cycle and pregnancy on activity of salivary ALDH3A1. As auxiliary parameters we also measured major salivary antioxidants, salivary peroxidase (SPO) activity and uric acid (UA) concentration.

2. Materials and methods

Fasting saliva samples were collected once from 63 women with uncomplicated pregnancies between the 5th and 39th weeks of gestation, aged 19–38 and from 39 healthy women of reproductive age, but not pregnant, aged 23–35. Women were selected from those who visited hospital outpatient clinic for a routine follow-up. The control and examined group consisted of women, who do not smoke or drink alcohol and had no symptoms of gingivitis nor periodontitis. All women with metabolic disease (diabetes, hyperlipidaemia) and receiving oral contraceptives were not included in the analysis. Saliva samples were also collected from 10 healthy women with regular menstrual cycles (27–30 days) and normal BMI (19–25 kg/m^2) four times during one menstrual cycle – in the early follicular (day 4–5), the mid-cycle (day O^{-1} – the day of the positive ovulatory test and day O – one day after the positive ovulatory test) and the luteal phase (day 21). The ovulation was predicted by urinary hormone measurements with the one step ovulation (LH) test strip (AI DE Diagnostic Co., Ltd., Chengyang district).

Resting whole saliva samples were collected between 8 and 9 am, after rising mouth with water, directly to empty test tubes (SPO, UA assay) or test tubes containing 50 mM pyrophosphate buffer, pH 8.1, 1 mM GSH and 1 mM EDTA (ALDH assay). Saliva was spun and the supernatant carefully collected and stored in ice. Protein content in the saliva supernatant was measured using Bradford reagent (Sigma).

2,7-Dichlorofluorescein, leuco-2,7-dichlorofluorescin diacetate, disodium phosphate (V), tetrasodium pyrophosphate (V), NAD$^+$, EDTA (ethylenediaminetetraacetic acid, disodium salt), 6-methoxy-2-naphthaldehyde, 6-methoxy-2-naphthoic acid and Bradford reagent were purchased from Sigma–Aldrich. Ethanol (99.8%), sodium hydroxide (NaOH), 30% aq. hydrogen peroxide (H_2O_2) and potassium thiocyanate (KSCN) were obtained from Chempur.

The ''Uric Acid liquicolor Plus'' uric acid assay kit was supplied by Human. Water was filtered through the Millipore utrafiltration system. All chemicals were of analytical grade. The applied buffers were devoid of any fluorescent impurities. A Nicolet Evolution 300 spectrophotometer was used to measure absorbance. Fluorimetric measurements were made using a Hitachi F7000 spectrofluorimeter.

ALDH3A1 activity was determined fluorimetrically, as described previously.[3] Briefly, fluorimetric assays were run in the 50 mM pyrophosphate buffer, pH 8.1, at 25°C, in the presence of 1 mM EDTA and 1 mM GSH (''real'' activity) or 0.5 mM DTT (total activity). The assays utilised a highly fluorogenic naphthaldehyde substrate, 6-methoxy-2-naphthaldehyde (5 μM), reacting with NAD$^+$ (100 μM) as a co-substrate. 6-Methoxy-2-naphthoic acid (1.5 μM) was added as internal standard.

The saliva samples were diluted 50-fold with the buffer, and an increase in the fluorescence of the naphthoate was recorded at 360 nm, with excitation at 315 nm, for 6–10 min. One enzyme unit is defined as the amount that oxidises 1 micromole of 6-methoxy-2-naphthaldehyde per minute. This unit is approximately twice as large as the commonly used benzaldehyde unit.

Salivary peroxidase (SPO) activity was determined fluorimetrically using the method reported by Proctor[11] and by utilising the enzymatic oxidation of the fluorogenic leuco-2,7-dichlorofluorescin (0.2 μM) to 2,7-dichlorofluorescein in the presence of 0.18 mM H_2O_2 and 1 mM KSCN at 67 mM phosphate buffer, pH 6. The typical saliva dilution in the cuvette was 150-fold. The reaction was performed at 25 °C. The method was modified by measuring the fluorescence slopes, dF/dt, rather than the fluorescence increase (dF), and by using the purified 2,7-dichlorofluoresceine as the internal standard to determine the absolute reaction rates.

Table 1 – Average values and dispersion of salivary aldehyde dehydrogenase (ALDH3A1), salivary peroxidase (SPO) activity and uric acid (UA) concentration during the menstrual cycle.

N = 10	Follicular phase		Mid-cycle				Luteal phase	
			Day O^{-1}		Day O			
	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)
Total ALDH3A1 activity[NP] [U/g]	1.5 (1.2)	1.0 (0.74–1.9)	3.7 (4.8)	1.7[a] (0.97–5.4)	1.8 (1.9)	1.0[a] (0.51–2.1)	2.8 (2.4)	2.3[a] (1.4–3.1)
''Real'' ALDH3A1 activity[NP] [U/g]	0.61 (0.61)	0.39 (0.12–0.80)	1.7 (2.4)	0.8[a] (0.23–2.1)	0.8 (1.1)	0.27[a] (0.14–0.85)	0.71 (0.78)	0.54[a] (0.16–0.72)
SPO activity[NP] [U/g]	0.62 (0.52)	0.56[a] (0.20–0.90)	0.68 (0.54)	0.58 (0.28–0.78)	0.68 (0.68)	0.64[a] (0.28–0.96)	0.32 (0.20)	0.28 (0.14–0.44)
UA concentration[P] [mmol/g]	0.21 (0.10)	0.22 (0.11–0.28)	0.26 (0.12)	0.22 (0.12–0.32)	0.22 (0.10)	0.22 (0.12–0.28)	0.38 (0.26)	0.38 (0.24–0.51)

[a] Shapiro–Wilk normality test rejected the hypothesis that variables in analysed group were normally distributed; therefore, medians are better estimators of expected values.
[P] Parametric hypothesis test was used.
[NP] Non-parametric hypothesis test was used.