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Effect of different storage conditions on the nitrite levels of human saliva

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Nitric oxide (NO) is an intracellular messenger molecule that plays an important role in biological systems as a physiological and pathophysiological mediator. Therefore, levels of NO in biological fluids reflect physiological aspects of diagnostic and therapeutic significance. Relatively stable end products of NO, nitrite and nitrate, is commonly measured to evaluate the production of NO in biological fluids such as serum, saliva and urine. Salivary nitrite and nitrate levels have been reported to reflect a spectrum of the health and disease states serving as non-invasive, clinically informative and effective source for prognosis, laboratory or clinical diagnosis in humans. However, detection of salivary nitrite levels in resource limited settings present several challenges such as availability of analytical equipment and stability of nitrite levels during sample storage and transportation. Hence, the aim of this study was to detect the effect of different storage conditions on the salivary nitrite levels to evaluate the stability of salivary nitrite during storage.

Saliva samples were collected from six healthy females between the age of 20-30 using the spit method. Salivary nitrite levels were between 8 - 46 μM (23.3 ± 10.4 μM) for samples that were analyzed directly after sample collection by Griess colorimetric reaction following stabilization with NaOH and deproteination with $ZnSO_4$. Samples from each individual was sampled twice. Similarly, the nitrite levels of the saliva samples were measured following storage for one hour at room temperature (RT) and at $4^{O}C$, and after storage overnight at RT and at -80 ^{O}C. Sample storage for one hr at RT (21.5 ± 5.1 μM) and at $4^{O}C$ (18.1 ± 4.6 μM) and overnight at -80 ^{O}C (22.0 ± 5.2 μM), prior to sample analysis did not show statistically significant difference in salivary nitrite levels from the direct sample analysis. Storage of samples overnight, at RT (3.6 ± 0.7 μM) prior to sample analysis, on the other hand, showed statistically significant difference ($P < 0.005$) in salivary nitrite levels compared to the nitrite levels detected during direct sample analysis based on student's t-test. The study reveals that the levels of nitrite changes during prolonged storage at room temperature while storage at ultralow temperatures is suitable for prolonged sample storage for subsequent analysis for salivary nitrites.

Keywords: Salivary nitrite, Griess reaction, Salivary biomarker

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