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Concentration of high-sensitivity cardiac troponin I in the oral fluid in patients with acute myocardial infarction: a pilot study

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Aim. To assess the potential of using oral fluid as a non-invasive diagnostic material in patients with myocardial infarction (MI).

Material and methods. The pilot, single-center, prospective study included 47 patients with documented MI, among whom there were 33 men (71%) and 14 women (29%) (mean age, 61,72±12,09 years). All patients successfully treated with reperfusion therapy. The control group consisted of 15 people in whom MI was not confirmed. The concentration of high-sensitivity cardiac troponin I (hs-cTnI) in blood and oral fluid was determined using chemiluminescence enzyme immunoassay (CLEIA) on a PATHFAST analyzer (LSI Medience Corporation). Medium sensitivity cardiac troponin I (ms-cTnI) was determined in blood using an Access 2 immunoassay system analyzer (Beckman Coulter, USA). Levels of total bilirubin, creatinine, glucose, rheumatoid factor, alkaline phosphatase and other biochemical parameters were determined on a Furuno CA-400 analyzer (Japan).

Results. The levels of hs-cTnI in patients with MI were significantly higher than in healthy patients both in blood (8,73±1,17 ng/ml vs. 0,012±0,03 ng/ml, p<0,001) and oral fluid (0,41±0,11 ng/ml vs. 0,004±0,001 ng/ml, p<0,001). In patients with AMI, there was a moderate correlation between the concentration of hs-cTnI in the serum and the oral fluid (r=0,319; p<0,05).

The serum level of hs-cTnI in patients with Q-wave (n=33) and non-Q-wave (n=14) MI was 10,11±1,53 ng/ ml vs. 5,48±1,29 ng/ml, respectively (p=0,025). The oral fluid concentration of hs-cTnI in patients with W-wave (n=33) and non-Q-wave (n=14) MI was 0,42±0,14 ng/ml vs. 0,40±0,16 ng/ml, respectively (p=0,925).

The serum level of hs-cTnI in anterior MI (n=19) was 8,92±2,06 ng/ml vs. 8,91±1,81 ng/ml in posterior one (n=23) (p=0,997). The concentration of hs-cTnI in the oral fluid was 0,21±0,06 ng/ml vs. 0,57±0,21 ng/ml, respectively (p=0,107).

The oral fluid concentrations of hs-TnI in patients with MI using conventional plastic tubes (n=26) and special Sarstedt microtubes (n=21) were 0,56±0,19 ng/ml and 0,22±0,10 ng/ml, respectively (p=0,12).

Conclusion. This pilot study has proven the possibility of detecting hs-cTnI in the oral fluid of patients with MI. There was a moderate correlation between the level of hs-cTnI in blood serum and oral fluid. Further research is needed to determine the hs-cTnI reference values in the oral fluid of patients with MI.

Keywords: high-sensitivity cardiac troponin I, myocardial infarction, non-invasive diagnostics, oral fluid.

Relationships and Activities: none.

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Cardiac specific troponins I and T are considered to be the best among all known biomarkers of myocardial damage in terms of sensitivity and specificity [1, 2]. Troponin, whose detection in blood serum was previously considered a key sign of myocardial infarction (MI), can now be regarded as a normal metabolite, since it can be determined even in blood serum of healthy individuals, if a highly sensitive technique is used [2].

Interest in studying the diagnostic potential of oral fluid has been recently shown by specialists in different fields, such as endocrinology, nephrology, dentistry and others [3, 4]. Human saliva is a dynamic biological fluid found mainly in the oral cavity. It is produced mainly by large paired salivary glands (parotid, sublingual, and submandibular), as well as, to a much lesser extent, by minor salivary glands. It is necessary to distinguish saliva from oral fluid. Oral fluid ("mixed saliva") should be considered a mixture of the overall secret of all salivary glands, microorganisms and products of their vital activity, gingival fluid, food residues, etc., while saliva is the secret produced by salivary glands only [4, 5]. The key function of oral fluid is to provide an optimal (satisfactory) environment for the healthy functioning of oral tissues, acting as a buffer lubricant and maintaining the ion reservoir, as well as to assist the normal course of the initial stages of digestion. The property of oral fluid that makes it so valuable to clinicians is that its composition reflects to some extent the levels of biomarkers present in the blood serum that clinicians rely on in diagnosis. Proteomic studies have revealed more than 1 thousand proteins and 19 thousand unique peptide sequences in the oral fluid, many of which have recently been in the focus of studies [3, 6]. The undoubted advantages of oral fluid over blood serum as a biological material can be considered non-invasive, non-traumatic and painless sampling procedure which does not require trained personnel.

Taking into account numerous reports of successful use of oral fluid for the diagnosis of various diseases, we decided to study the diagnostic value of high-sensitive troponin I (hs-TnI) in oral fluid of patients with MI. To address this task, the study was divided into several consecutive stages. At the first stage, which is presented in this article, we planned to investigate the very possibility of detecting elevated levels of hs-TnI in oral fluid of patients with MI.

Thus, the goal of the research was to study the possibilities of using oral fluid as non-invasive diagnostic material in patients with acute MI.

Material and methods

The pilot single-center prospective study was carried out according to the Good Clinical Practice and the principles of the Helsinki Declaration. Patients who participated in the study signed an informed consent form.

The entry criteria were as follows: patients of any gender and age; confirmed MI (time interval of 12 to 24 hours from the moment of hospitalization); myocardial revascularization (percutaneous coronary intervention or thrombolytic therapy); voluntary consent of the patient to participate in the study. Exclusion criteria: chronic kidney disease (CKD) and other pathological conditions that cause a non-coronarogenic increase of cardiac troponins (cardiomyopathies; myocarditis; false-positive interferences of heterophilic antibodies, alkaline phosphatase, bilirubin, rheumatoid factor and other agents).

Venous blood was taken from all the patients by antecubital venipuncture. After that, patients were asked to donate oral fluid by spitting for 1-2 minutes, so that some patients collected oral fluid into ordinary plastic tubes, and others — into Sarstedt micro-tubes (Sarstedt, Germany), specially designed for saliva collection. In this fashion, the diagnostic strength attained with different types of test tubes could be assessed and compared.

Biological material (blood and oral fluid) was delivered to the laboratory for further sample preparation and determination of moderate-sensitive troponin I (ms-TnI) and hs-TnI, as well as a number of biochemical parameters. Blood and oral fluid samples were centrifuged at 3000 rpm for 5 minutes to obtain the supernatant fluid. Some samples of oral fluid (with increased viscosity) were additionally centrifuged to ensure the physical state suitable for analysis.

The determination of Hs-TnI in oral fluid and blood serum was carried out using the PATHFAST rapid compact chemiluminescent immunoassay analyzer (LSI Medience Corporation), and the concentration was expressed in nanograms per milliliter (ng/ml). The principle of the method for the determination of cardiac troponin I is based on chemiluminescent enzyme immunoassay (CLEIA) and consists of several phases: at the first stage, diagnostic antibodies labeled with alkaline phosphatase interact with epitopes of the troponin I molecule; at the second stage, a luminescent substrate is introduced, which is fermented with alkaline phosphatase, which leads to the emission of photons, the intensity of which is detected by a photomultiplier. The signal intensity is directly proportional to the number of diagnostic antibodies bound to the desired antigen (troponin I), and the concentration is calculated from the calibration curve.

Blood serum Ms-TnI was determined using the Access 2 automatic immunoassay analyzer (Beckman

Table 1

Clinical characteristics of groups of examined patients*

Parameter/test	MI patients (n=47)	Control group (n=15)
Age, years	61,72±12,09	57,1±10,2
Sex (male)	33 (71,3%)	7 (46%)
Hypertension (abs.; %)	44 (93,6%)	9 (60,0%)
Prior MI (abs.; %)	10 (21,2%)	2 (13,3%)
Prior cerebrovascular insult (abs.; %)	3 (6,3%)	2 (13,3%)
Peripheral vascular disease (abs.; %)	9 (19,1%)	4 (26,6%)
CHF, FC III-IV (abs.; %)	6 (12,7%)	3 (20,0%)
Smoking (abs.; %)	8 (17,0%)	2 (13,3%)
Diabetes mellitus (abs.; %)	9 (19,1%)	1 (6,6%)
Morphine (abs.; %)	29 (61,7%)	-
Beta-blockers (abs.; %)	47 (100%)	13 (86,6%)
ACE inhibitors (abs.; %)	43 (91,4%)	9 (60%)
Statins (abs.; %)	47 (100%)	9 (60%)
Aspirin (абс.; %)	47 (100%)	9 (60%)
Thrombolytic therapy (abs.; %)	7 (14,8%)	-
Percutanenous coronarography intervention (abs.; %)	40 (85,2%)	-
Increased ms-TnI in blood serum (abs.; %)	47 (100%)	-
Glucose, μmol/L	5,22±0,31	4,62±0,46
Creatinine, μmol/L	109,34±10,82	105,42±9,61
Glomerular filtration rate, mL/min /1,73 m^2	79,11±11,08	83,26±14,15
Rheumatoid factor, IU/mL	5,67±4,56	4,88±3,74
Total bilirubin, μmol/l	12,35±4,57	14,86±3,89
Alkaline phosphatase, U/L	123,48±39,72	118,31±28,01
MI with Q-wave (abs.; %)	33 (70%)	-
MI without Q-wave (абс.; %)	14 (30%)	-
Anterior MI (abs.; %)	19 (40,4%)	-
Posterior MI (абс.; %)	23 (49,0%)	-
MI of unspecified site (abs.; %)	5 (10,6%)	-

Note: * — no significant inter-group differences in any parameter.
Abbreviations: ACE — angiotensin-converting enzyme, MI — myocardial infarction, FC — functional class, CHF — chronic heart failure.

Table 2

Concentration of hs-TnI in patients with acute MI and in control group

	MI patients (n=47)	Control group (n=15)	P
hs-TnI, ng/mL (in blood serum)	8,73±1,17	0,012±0,03	p<0,001
hs-TnI, ng/mL (in oral fluid)	0,41±0,11	0,004±0,001	p<0,001

Note: M — arithmetic mean value, m — arithmetic mean error, p — significance level attained.
Abbreviations: hs-TnI — high-sensitive troponin I, MI — myocardial infarction.

Coulter, USA). Biochemical parameters (glucose, creatinine, rheumatoid factor, alkaline phosphatase, total bilirubin) were measured using the Furuno CA-400 automatic analyzer (Japan).

The obtained data were analyzed using the Statistica 7.0 package. The results were expressed as M±m (arithmetic mean and arithmetic mean error, respectively). Case-control groups were compared using unpaired and independent Student t-tests. Relationships (correlations) were studied using Pearson correlation analysis. The critical significance level, p, was chosen to be 0,05.

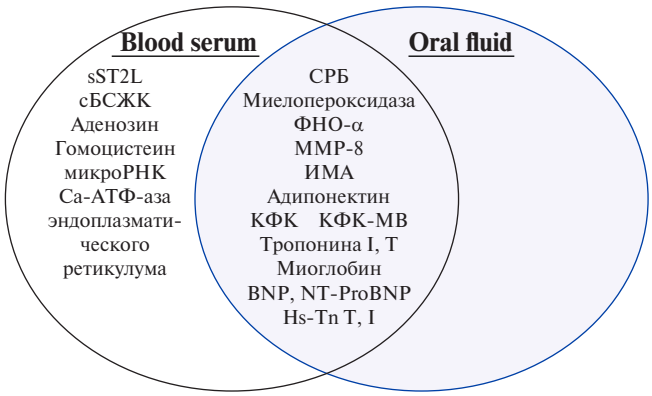

Figure 1. Basic cardiac biomarkers found in blood serum and oral fluid [2].
Abbreviations: ATP — adenosine triphosphate, IMA — ischemia modified albumin, CPK — creatine phosphokinase, CPK-MB — creatine phosphokinase-MB, CRP — C-reactive protein, CVD — cardiovascular disease, h-fabp — heart-type fatty acid binding protein, TNF-α — tumor necrosis factor α, MMP-8 — matrix metalloproteinase-8, BNP — brain natriuretic peptide, NT-proBNP — N-terminal pro B-type natriuretic peptide, sST2L — isoform of ST2 protein, Hs-Tn — high-sensitive troponin.

Results

The study included 62 patients. The main group included those with confirmed MI diagnosis (n=47), and the control group consisted of patients (n=15) hospitalized with other diseases (prior MI, stable angina pectoris, chronic heart failure, cardiac arrhythmias, hypertension). The clinical profile of the patients is presented in Table 1.

Patients of the main and control groups did not differ in age significantly, but men predominated among patients of the main group (71,3%). Creatinine levels and estimated glomerular filtration rate did not differ significantly between the groups. The absence of an increase in the level of a number of biochemical parameters (rheumatoid factor, total bilirubin, alkaline phosphatase) allowed us to practically exclude false-positive interference. In general, the levels of biochemical parameters (glucose, creatinine, rheumatoid factor, total bilirubin and alkaline phosphatase) did not differ significantly between the groups.

Myocardial revascularization was performed in all patients. MI with Q-wave was formed in 70% of patients, and 30% were without Q-wave.

In all 47 patients of the main group, ms-TnI level was higher than the upper reference limit (>0,4 ng/mL), while in the control group it was not detected (0,00 ng/mL) or its values were below 0,02 ng/mL.

Serum hs-TnI level in MI patients was also many times higher than the threshold value (99th percentile is >0,015 ng/mL for our test system; to convert to common measurement units, ng/L must be multiplied by 1000, which gives 15 ng/L) and amounted to 8,73±1,17 ng/mL. In patients of the control group (n=15), the serum hs-TnI concentration was 0,012±0,03 ng/mL, the significance level for intergroup differences being p<0,001.

The oral fluid of patients with confirmed MI showed significantly higher hs-TnI level compared to that in the control group: 0,41±0,11 ng/mL vs. 0,004±0,001 ng/mL (p<0,001) (Table 2). A moderate correlation was found between the concentration of hs-TnI in blood serum and oral fluid in patients with MI (r=0,319; p<0,05).

Serum levels of hs-TnI in patients with MI with Q-wave (n=33) and without it (n=14) were 10,11±1,53 ng/mL vs. 5,48±1,29 ng/mL, respectively (p=0,025). Concentrations of hs-TnI in the oral fluid in patients with MI with Q-wave (n=33) and without Q-wave (n=14) were 0,42±0,14 ng/mL vs. 0,40±0,16 ng/mL, respectively (p=0,925).

The level of hs-TnI in blood serum in patients with the anterior MI (n=19) was 8,92±2,06 ng/mL vs. 8,91±1,81 ng/mL for the posterior MI (n=23) (p=0,997). The concentration of hs-TnI in the oral fluid is 0,21±0,06 ng/mL vs. 0,57±0,21 ng/mL for the anterior and posterior MI, respectively (p=0,107).

Concentrations of hs-TnI in the oral fluid of MI patients using conventional plastic tubes (n=26) and special Sarstedt micro-tubes (n=21) were 0,56±0,19 ng/mL and 0,22±0,10 ng/mL, respectively (p=0,12).

Discussion

To date, most of the biomarkers of cardiovascular diseases present in blood have also been identified in oral fluid (Figure 1) [2]. However, the number of studies devoted to the non-invasive determination of cardiac biomarkers is still relatively small; therefore, further research is needed.

Our findings of an increase in the level of hs-TnI in the oral fluid of MI patients are consistent with studies by other authors [7-10]. A group of American researchers headed by Floriano PN (2009) was the first to identify >20 biomarkers, including troponin I, in the oral fluid of MI patients using the lab-on-a-chip nanotechnology (LOC) [7]. Subsequently, Miller CS et al. (2010) found elevated levels of myoglobin in the oral fluid of MI patients [8]. Mirzaii-Dizgah I et al. (2013) conducted a number of studies in which biomarkers of MI (creatine kinase MB-fraction and cardiac troponins T and I) were determined in the oral fluid of patients using enzyme-linked immunosorbent assay [9, 10].

However, the currently available results are not sufficient for using in clinical practice. First of all, it should be noted that there are no specific reference values for the levels of cardiac troponins

in other biological fluids, particularly in oral fluid, due to the relatively small number of studies [2]. Troponin levels measured in oral fluid are different in different researchers. The obvious discrepancy in concentrations obtained in different studies can be explained by the use of different commercial setups and methods for the determination of concentrations of troponins. Diagnostic antibodies that are part of immunoassays differ in that they are aimed at different antigenic determinants of the molecules of troponins. In other words, different test systems for the determination of troponins detect different epitopes of molecules of the heterogeneous troponin fraction: free troponin molecules, troponin complexes, troponin fragments of different sizes and molecular weight, as well as their oxidized and phosphorylated derivatives [2]. In the present study, a highly sensitive chemiluminescence enzyme immunoassay was used, in contrast to the studies by Floriano PN (2009) and Mirzaii-Dizgah I (2013), where LOC and enzyme-linked immunosorbent assay were used [7, 9, 10].

In the present study, it was found that the serum level of hs-TnI in MI patients with Q-wave was significantly higher than that in MI patients without Q-wave (p=0,025), but the respective differences in oral fluid were not statistically significant. The localization of ischemic damage did not substantially affect the result: there were no significant differences of either serum or salivary levels of hs-TnI between patients with anterior and posterior infarction. It is very likely that this is due to the phenomenon of leaching of troponins from the necrotic zone, which is determined by the degree of reperfusion, as well as by many other parameters, such as the time of admission of patient and the time of sampling of biological material, method used for the determination of troponins, and others. Thus, according to the study by Chia S. et al., the best correlation of troponin level with the size of infarction was observed 72-96 hours after the onset of ischemic symptoms for patients who underwent percutaneous coronary intervention [11]. In our study, however, the biological material was taken 12-24 hours after the admission of patients. In addition, we found no significant differences in the measured levels of hs-TnI in the oral fluid of patients when different test tubes were used for sampling.

Human biological fluids, including oral fluid, are considered ultrafiltrates of blood plasma, and therefore contain almost the same components, but in different ratios, which is primarily due to the mechanisms of their transport (filtration) [2, 10, 12]. Mechanisms of transport of troponins to other biological fluids through blood-tissue barriers are debatable. So, for example, according to some researchers, troponins are too large protein molecules to pass through the glomerular and blood-saliva filters (barriers). Indeed, in Ziebig R, et al. (2003), in most MI patients, troponin I was not detected in urine, and the authors concluded that cardiac troponins, due to their size, cannot pass through the glomerular filter [12, 13]. At the same time, patients with CKD may have elevated levels of troponins even in the absence of coronary pathology. Moreover, in patients with more severe CKD (more pronounced inhibition of glomerular filtration), troponin levels were higher than in patients with initial stages of CKD, and therefore a number of researchers give the kidneys a leading role in the elimination of troponins from the blood [12]. In addition, a recent study found troponin I in the morning urine of all healthy patients as well as patients with high blood pressure; moreover, troponin I concentrations in patients with hypertension were significantly higher than in normotonic patients. This can be considered the direct evidence of the role of kidneys in the elimination of troponins. Probably the success of this study [14] can be ascribed to the use of highly sensitive immunoassay, in contrast with the study by Ziebig R [13], who used a moderately sensitive system, whose detection ability was not enough to detect such relatively low concentrations of troponin I.

One of the factors contributing to the filtration of troponins through narrow pores is intra- and extracellular degradation into smaller fragments. According to current data, several tens of fragments of various sizes and molecular weights are found in blood serum. Apparently, smaller fragments can penetrate the blood-tissue interface, as evidenced by numerous studies in which troponins were found in urine [14], cerebrospinal fluid [15] and oral fluid [2, 7, 9, 10]. These fragments are though “visible” only to those test systems that contain antibodies specific to them.

Another important factor affecting the diagnostic strength of all laboratory tests such as oral fluid studies is the preanalytical stage, which largely depends on how accurately all recommendations regulating the sampling of biological material and the work of health care personnel are followed. According to current data, the most frequent errors related to incorrect test results are caused by preanalytical mistakes in 70% of cases. The preanalytical stage, including the sampling conditions and the details of sample preparation, is also not standardized and is different in different researchers, and this could of course have affected the results.

Among the limitations of this method for the determination of troponin I are that the result can be influenced by the state of the oral mucosa and concomitant (background) dental diseases, the time

of eating, the mode of teeth brushing and rinsing the oral cavity, saliva collection speed. In our future studies, we are planning to take these points into account to improve the effectiveness of the use of oral fluid for the diagnosis of MI.

Conclusions

The present pilot study has proved that it is possible to detect hs-TnI in the oral fluid of patients with confirmed MI. There is a moderate correlation between the levels of hs-ThI in the blood serum and oral fluid, which may be due to the characteristics of the preanalytical stage or the state of the oral mucosa. Further studies are needed to determine the reference values for the hs-ThI content in the oral fluid in MI.

Relationships and Activities: none.

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