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A Comparison Between Two Assays for the Redox Status in Plasma

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Abstract

In the present study, two commercial assays for the redox status are compared and evaluated, the SH groups in proteins (SHp) and the total thiol levels (TTL). Both assays are colorimetric endpoint reactions and have been adapted for use on an automatic clinical analyzer. Both assays performed well by giving comparable results in plasma samples. A good correlation with a correlation coefficient (R^2) of 0.889 was found between the two assays. However, for some pathological specimens, especially highly lipemic samples, the SHp assay performed better than the TTL assay. When plasma samples with a high lipemic index were omitted, the correlation between the two assays was even better with an R^2 of 0.942.

In conclusion, both assays for the redox status performed well, but the SHp assay can also be used for high lipemic samples.

Keywords: Colorimetry; Redox; Human serum; Lipemic index

Introduction

The redox status is an important tool in determining the physiological state of the body, especially for the elderly and with hypertension and heart failure [1-4]. A reflection of the redox status in the circulation can be measured by the level of free thiol groups in proteins in serum or plasma samples. There are several assays described in the literature for the evaluation of the redox status in serum or plasma. In these simple assays, a reaction of thiol groups of proteins takes place with a chromogen. The most used assay is based on the reaction of 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), with thiol groups which can be easily performed with home-made materials [5,6]. However, for quality control during large-scale studies, commercially available assays are preferred due to the long-term delivery and stability of the assay components and the availability of quality control materials. This test can be performed either by high performance liquid chromatographic methods [7-9] or in micro plate format [10] and was also programmed and applied to clinical analyzers [11-14]. The use of auto-analyzers allows measurement of large sets of samples in epidemiological studies under the required quality during longer periods. Another advantage is the possibility to combine the assay with the measurement of other biomarkers in the same small volume of sample [15].

In the present paper, we have compared the performance of two commonly used assay systems from two commercial companies which are based on the DTNB reaction. The performance was tested in a number of human plasma samples from a clinical study on hypertension.

Materials and Methods

Blood was collected of sixty hypertensive patients which were included in the BEST-IN-TENSION Registry (Benefit for diaSTolic heart failure from Intensified management of refractory hyperTENSION), located at the Comprehensive Heart Failure Center in Würzburg. A number of 17 controls were included from the MyStIC (Myocardial Sodium content In Cardiac disease) study. Informed consent was obtained from all participants.

The blood samples were processed for EDTA-plasma and aliquots were stored at -80°C until analysis. The measurements of the total thiols in proteins were carried out with the SHp and the TTL test. Both methods are rather similar and based on the reaction of protein thiols with DTNB, giving a colored reaction product with an absorbance maximum at 412 nm. The results of the tests have been expressed in µmol/L. Both tests have been adapted for use in the D×C 800 clinical auto-analyzer (Beckman-Coulter, Woerden, the Netherlands). The SHp test (SH groups in proteins) was obtained from Diacron, Grosseto, Italy. The TTL assay (total thiol levels) was obtained from RelAssay, Gaziantep, Turkey. All data have been performed as single measurements. The intra-assay variation (%) of each assay was determined with two control materials. A routinely used human serum sample and a quality control (QC) sample from each of the manufacturers. The coefficient of variation (CV) for the SHp assay was 2.6% (at a level of 777 µmol/L) and 3.1% (at a level of 466 µmol/L) as determined with both samples, respectively (n=9). The coefficient of variation (CV) for the TTL assay was 2.5% (at a level of 623 µmol/L) and 2.8% (at a level of 1027 µmol/L) as determined with both samples, respectively (n=9).

Results

Both of these assays for the redox status gave similar results with respect to the reproducibility. The intra-assay coefficients of variation were almost similar for both assays. The average CV was 2.9% for the SHp assay and 2.7% for the TTL test. When all samples were included, the correlation between the two assays was rather good. In Figure 1, the correlation for all samples is shown. The correlation coefficient (R^2) is 0.889 with the equation of the correlation curve of 'y=0.86x-49'. This means that the SHp assay exhibited slightly higher average concentrations (14% higher) than the TTL assay, probably due to the calibration of both commercial assays.

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Both of these assays for the redox status were adapted for use in a clinical auto-analyzer to be able to conduct large series of measurements of serum or plasma samples in epidemiological studies [8]. The auto-analyzer also measured three different indices for the assessment of the quality of the plasma samples, the hemolytic, icteric and lipemic index. Each index has an arbitrary range from 0 (low disturbance) to 10 (high disturbance). Each biomarker has for each of the three indices a certain cut-off value, above which the result of the measurement of the biomarker is no longer reliable. It appeared that both redox assays were not very sensitive to the hemolytic and icteric index, but rather sensitive for the lipemic index. The higher the lipemic index, the lower the measurements of the redox status. This is shown in Figures 2 and 3.

In Figure 2, the lipemic indices were plotted against the measurements with the SHp assay of all plasma samples. It turned out that there was a slight reduction of SHp levels with increasing lipemic index values with a slope of -7.98. The p-value of the regression line was 0.244 (non-significant).

For the TTL assay, a much larger deviation was observed at higher values of the lipemic index as shown in Figure 3. Here the slope is -25.8 which means a substantial reduction of the TTL values with increasing lipemic index. Here the p-value of the regression line was 1.7*E-5

If the plasma samples with lipemic index greater than 3 were left out of the analysis, a much better correlation with a correlation coefficient

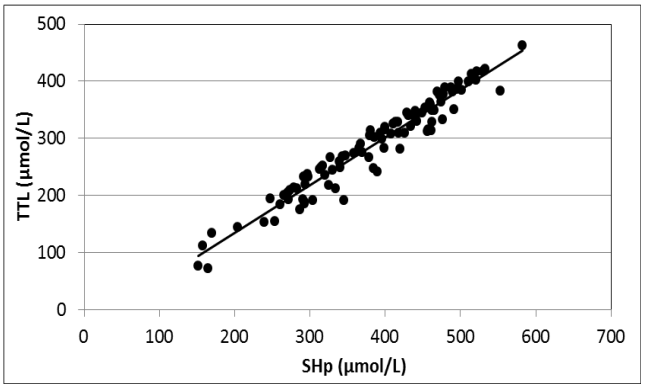

Figure 1: Correlation between two assays of the redox status: SHp and TTL using the data of all participants in this study.

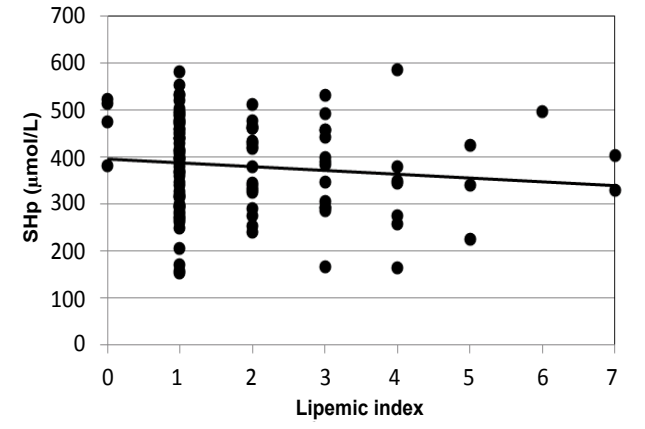

Figure 2: A plot of the redox status as measured by the SHp assay against the lipemic index of the samples. A linear regression line was fitted through all data points with the equation 'y=-7.98x+395'.

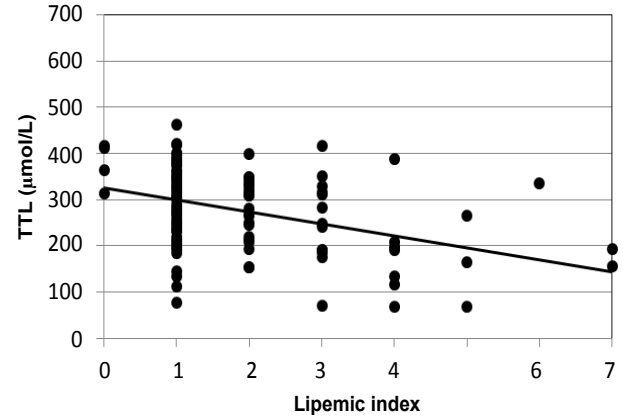

Figure 3: A plot of the redox status as measured by the TTL assay against the lipemic index of the samples. A linear regression line was fitted through all data points with the equation 'y=-25.8x+325'.

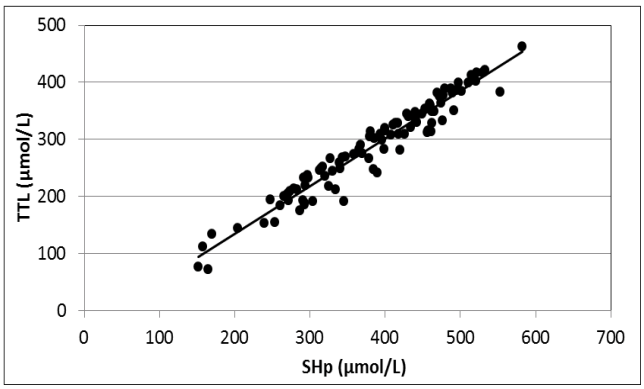

Figure 4: Correlation between two assays of the redox status: SHp and TTL with the data of participants in this study with a lipemic serum index of ≤ 3.

(R^2) of 0.942 is found between the two assays with an equation of the correlation curve of 'y=0.84x-32.8' as is shown in Figure 4.

Discussion

The redox status is an important biomarker for the assessment of the general state of health in heart failure and elderly [16-18]. With increasing age the redox status decreases, making the elderly more prone for oxidative stress related processes [19]. The redox status was measured using a simple colorimetric assay based on a reaction of the free SH-groups of the cysteine side chains in proteins with DTNB. The assay performance is suitable for automated clinical analyzers. The assay determines all thiols, but in serum or plasma the protein thiols are the main components [20]. The assay showed a good storage stability of the human serum and plasma samples at -80°C for longer periods [21]. In addition, the short-term stability is also very good which makes it easier in day-to-day handling of samples [22].

In the present report, two commercial assays have been compared with respect to linearity and sensitivity to pathological samples. Both assays are not very sensitive to hemolytic and icteric samples. But with regard to lipemic samples, often a result of a high concentration of triglycerides, both assays behave differently. It was found that the SHp assay is less sensitive to the influence of lipemic samples compared to the TTL assay. A linear regression line plotted through data points against

the lipemic index, revealed a much larger decline in the TTL assay than in the SHp assay. The regression line of the TTL assay vs. the lipemic index for values from 1 to 7, showed a decay in the measurements of the TTL assay of 53% (statistically significant), whereas in the SHp assay this decrease is only 12% (statistically not significant), as shown in Figures 2 and 3.

It is concluded that both assays for the redox status are performing very well and that they can be applied in large-scale studies. The only difference is the better performance of the SHp assay with high lipemic plasma samples.

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