ENHANCING THE ACCURACY OF CERULOPLASMIN LEVEL DETERMINATION IN CLINICAL PRACTICE THROUGH PROTEOLYSIS INHIBITORS

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This article examines the impact of protease inhibitors on the accuracy of quantifying ceruloplasmin, a copper-containing protein critical for copper metabolism and disease diagnosis. Standard methods, such as ELISA, may yield elevated ceruloplasmin levels due to antibody interactions with protein structures. We investigated serum samples from patients with genetic disorders and applied protease inhibitors like PMSF. Results indicate that these inhibitors enhance ceruloplasmin stability and improve measurement accuracy, offering potential advancements in diagnostic methods.

Keywords: Ceruloplasmin, proteolysis, copper metabolism, diagnostic marker, ELISA, serum analysis, enzyme inhibitors.

Introduction. Ceruloplasmin (CP) is a multisubunit copper-containing protein that plays a crucial role in copper metabolism and functions as an essential marker for inflammatory processes in the body [8, p. 77-86]. As a major transport protein for copper, ceruloplasmin is responsible for delivering this vital trace element to various tissues, thus ensuring its availability for important biochemical reactions. In addition to its role in copper transport, ceruloplasmin also exhibits antioxidant properties, participating in the detoxification of reactive oxygen species [1, p. 1-23]. Elevated levels of serum ceruloplasmin have been associated with conditions such as Wilson's disease, rheumatoid arthritis, and liver dysfunction, making it a valuable biomarker in clinical diagnostics [2, p. 403-410].

Serum ceruloplasmin levels can fluctuate significantly in response to various pathological conditions, including genetic disorders, inflammatory diseases, and liver diseases. Hence, accurate quantification of ceruloplasmin levels is critical for timely diagnosis and effective monitoring of these diseases. A thorough understanding of the factors affecting ceruloplasmin concentration can enhance the clinical utility of this protein as a diagnostic marker [3, p. 1860-1866].

Various methods are currently employed in clinical practice to analyze ceruloplasmin levels, with enzyme-linked immunosorbent assay (ELISA) and biochemical spectrophotometry being the most commonly used techniques. These methodologies allow for efficient and sensitive detection of ceruloplasmin; however, they may yield inaccurate results due to the influence of proteolysing enzymes. Such enzymes can cleave the protein and alter its structural conformation, leading to erroneous over- or underestimation of ceruloplasmin levels [4, p. 590-596]. Recent advancements in the field of proteomics and a better understanding of proteolytic activities highlight the need for optimizing these analytical methods to improve the reliability of ceruloplasmin measurements. This article explores the potential impact of protease inhibitors in stabilizing ceruloplasmin and enhancing the accuracy of its quantification in serum samples [5, p. 77-84].

Purpose of the study. The aim of this study is to evaluate the effect of proteolysis inhibitors on the stability of the octameric ceruloplasmin molecule and their ability to prevent proteolytic cleavage in the quantification of SR levels. We hypothesise that the use of these inhibitors will not only improve the accuracy of the measurements, but also provide a better understanding of the mechanism of ceruloplasmin-antibody interaction.

Study Objectives. In order to achieve the objective, a number of tasks are required:

1. To evaluate the efficacy of different proteolysis inhibitors, such as PMSF (phenylmethyl-

sulfonyl fluoride) and a complex cocktail of inhibitors, in preventing proteolytic cleavage of ceruloplasmin.

2. Analyse the data to identify patterns of change in the stability of the ceruloplasmin molecule depending on the inhibitors used.

3. To compare the results of quantification of ceruloplasmin in serum samples with and without the use of inhibitors.

Materials and Methods.

The following materials were used in the study:

1. Object of study: 100 serum samples from patients with genetic disorders.

2. Protease inhibitors:

– cOmplete™, Mini Protease Inhibitor Cocktail Tablets (Roche);

– PMSF (phenylmethylsulfonyl fluoride, SIGMA, USA).

Preparation of inhibitor solutions

The process of preparing a working solution for PMSF involved dissolving 0.087 grams of the substance in 1 ml of isopropanol followed by stirring and heating to 42°C. The prepared solution was stored in a dark place. For the complex inhibitor cocktail, one tablet was dissolved in 1.5 ml of chilled saline solution or deionised water.

Statistical analysis. The collected data were processed using statistical methods to assess the accuracy and reliability of the results obtained. Standard statistical methods such as overall mean, standard deviation and regression analysis were used for the analysis, which allowed us to identify patterns depending on the effect of inhibitors on the stability of ceruloplasmin molecules.

Results. The results of our study are based on the evaluation of serum samples divided into three groups: a control group without inhibitors, a group with added PMSF and a group with a complex cocktail of inhibitors. As a result of the study, the obtained data of quantification of ceruloplasmin concentration showed the following results:

– Control group: Ceruloplasmin levels ranged from 23.3 to 29.7 mg/dl.

– Group with PMSF: The levels were significantly lower, ranging from 17.4 to 24.1 mg/dl, indicating the inhibitory properties of PMSF.

– Group with complex cocktail of inhibitors: Ceruloplasmin levels were also lower, but ranged from 20.9 to 27.2 mg/dl.

These data confirm that the addition of inhibitors such as PMSF and complex cocktail significantly affects the stability of the ceruloplasmin molecule and avoids proteolytic cleavage.

Discussion. The study showed that the use of proteolysis inhibitors is an effective method to improve the accuracy of quantification of ceruloplasmin levels in serum samples. The stabilisation of the protein structure achieved by inhibitors prevents protein cleavage and therefore reduces the likelihood of false results.

It should be noted that the choice of a particular inhibitor is also important. For example, in our work, PMSF provided a greater reduction in ceruloplasmin levels compared to the complex cocktail. This may be due to differences in the mechanisms of action of these inhibitors as well as their different ability to bind to proteases. This opens new possibilities for further studies aimed at optimising assay methods.

Conclusion. The results of this study confirm that the use of proteolysis inhibitors can significantly improve the accuracy of determining ceruloplasmin levels in clinical practice. This has important implications for the diagnosis and monitoring of diseases associated with copper metabolism disorders. Further studies are needed to confirm the universality of the results obtained and to consider the use of other proteolysis inhibitors to improve the accuracy of various assays.

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ПОВЫШЕНИЕ ТОЧНОСТИ ОПРЕДЕЛЕНИЯ УРОВНЯ ЦЕРУЛОПЛАЗМИНА В КЛИНИЧЕСКОЙ ПРАКТИКЕ С ПОМОЩЬЮ ИНГИБИТОРОВ ПРОТЕОЛИЗА

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В данной статье рассматривается влияние ингибиторов протеаз на точность количественного определения церулоплазмина, медьсодержащего белка, критически важного для метаболизма меди и диагностики заболеваний. Стандартные методы, такие как ИФА, могут давать завышенные уровни церулоплазмина из-за взаимодействия антител со структурами белка. Мы исследовали образцы сыворотки крови пациентов с генетическими заболеваниями и применяли ингибиторы протеазы, такие как PMSF. Результаты показывают, что эти ингибиторы повышают стабильность церулоплазмина и улучшают точность измерений, предлагая потенциальные усовершенствования в методах диагностики.
Ключевые слова: церулоплазмин, протеолиз, метаболизм меди, диагностический маркер, ИФА, анализ сыворотки, ингибиторы ферментов.