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Dkk-1 level detection in ankylosing spondylitis by the aptamer-based colorimetric assay

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Motivation and Aim: The pathological process in ankylosing spondylitis (AS) includes bone tissue remodeling with possible bone loss and the development of secondary osteoporosis. A set of proteins regulate pathological bone metabolism in AS. Among them, the soluble serum protein Dkk-1, an antagonist of the Wnt signaling pathway, is of particular interest and importance. The lack of a generally accepted standard method for Dkk-1 detection is an obstacle in research of its role in AS structural progression. Oligonucleotide aptamers specific to certain biomarkers are very prospective for developing novel diagnostic assays due to their high affinity and the possibility of reproducible, scalable and cost-effective chemical synthesis. Stable properties of aptamers provide high reproducibility of the assays, which is especially important for longitudinal studies. In this work, we aimed to compare methods for measuring Dkk-1 in blood serum using conventional ELISA and the aptamer/antibody assay and to investigate the relationship between Dkk-1 levels and AS structural progression and secondary osteoporosis.

Methods: The study included 17 patients with AS (5 at the advanced stage and 12 at the late stage) and 8 healthy volunteers. Serum samples were diluted 10-fold prior to analyses. Colorimetric microplate ELISA of Dkk-1 in blood serum was performed using a commercial ELISA Kit. For the aptamer/antibody assay, the TD10 DNA aptamer [1] to human Dkk-1 was chemically synthesized, purified, and covalently immobilized in the wells of a transparent 96-well DNA-BIND plate. In all cases, anti-Dkk-1 antibody with horseradish peroxidase and chromogenic substrate were used for specific visualization of the analyte. The data from colorimetric analyses were preliminarily checked for normality and then the nonparametric Mann–Whitney U-test was used. The difference was considered statistically significant at $p < 0.05$.

Results: The results of colorimetric detection of the Dkk-1 level by ELISA aptamer-based assays showed good coincidence. The data indicate a significant increase in the level of serum Dkk-1 in patients with AS compared with healthy donors. Notably, the analysis by the aptamer/antibody assay fairly distinguished the samples from healthy volunteers and from AS patients without structural radiographic progression. In patients with syndesmophytes, the levels of serum Dkk-1 exceeded those for patients without syndesmophytes. At the same time, measuring Dkk-1 levels by both methods did not reveal significant differences between the groups of patients with or without osteoporosis.

Conclusion: Serum levels of Dkk-1 in AS patients differed significantly depending on the disease stage, but did not depend on the presence or absence of osteoporosis. Dkk-1 represents a potential serum marker of AS progression, capable of reflecting a trend towards the structural progression of the disease before the appearance of characteristic radiographic changes. The DNA aptamer-based test system allows for measuring the level of Dkk-1 in the blood serum of AS patients. In terms of diagnostic significance, this assay is highly competitive with conventional ELISA and exceeds the latter by a number of benefits.

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References

1. Zhou Y. et al. Developing slow-off dickkopf-1 aptamers for early-diagnosis of hepatocellular carcinoma. *Talanta*. 2019;194:422-429.