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Waldenström macroglobulinemia with unusual migration of the monoclonal protein to ß1 region on Serum Protein Electrophoresis

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Abstract

Waldenström macroglobulinemia (WM) is a rare mature B-cell malignancy characterized by an infiltration of lymphoplasmacytic lymphoma in the hematopoietic bone marrow associated with the occurrence of a monoclonal immunoglobulin M (Ig M) in blood serum. Screening and measurement of monoclonal proteins in WM are commonly performed by Capillary Serum Protein Electrophoresis (CSPE). The presence of an IgM paraprotein in the beta-1 fraction is unusual and can be challenging for monitoring patients since it can be obscured by the transferrin, protein regularly present in this fraction. Here we report a case of WM with an unusual migration of the Ig M monoclonal protein to the beta-1 region on CSPE and we discuss the difficulties his implies for the quantification of monoclonal protein by electrophoresis when measurements are contaminated by normal serum proteins in this region.

Keywords: Waldenström macroglobulinemia; beta-1fraction; monoclonal gammopathy; capillary serum protein electrophoresis

Case study *Corresponding Author, e-mail: aboulmakarimsiham@outlook.com

1. Introduction

Capillary zone electrophoresis (CZE) is an analytical tool for separating serum proteins on the basis of molecular size, electric charge, and hydrophobicity [1]. This technique requires the use of a high concentration of a basic buffer to negatively charge the proteins and reduce the interactions between the capillary walls and the proteins [2], proteins are detected directly using UV absorption [3]. Serum proteins are separated by CZE into six major fractions: albumin, alpha-1, alpha-2, beta-1, beta-2 and gammaglobulins, changes in the shape or amplitude of these fractions can point the clinician to a wide range of pathologies

[2]. Much of the clinical interest of CZE is the detection of monoclonal components (M-protein) which are usually referred to as an additional peak. The gamma region is the area where the majority of monoclonals migrate and immunoglobulins are usually the only proteins present [4]. Nevertheless, the migration of a monoclonal component in the alpha-2, beta-1, or beta-2 fraction is challenging because it can be masked by other proteins physiologically present in this fraction. The presence of monoclonal proteins in the beta fraction is often of the Ig A [5] or free light chain type. When a monoclonal component is co-migrating in the beta region, it's referred to as an additional peek, a single, a totally integrated beta peak, the concentration of the beta fraction is variable depending on its concentration [6]. It's important to check any sample with a localized peak or asymmetric distribution on SPE with immunofixation or any other technique confirming and characterizing the heavy and light chains [7]. The measurement of monoclonal protein of the immunoglobulin M paraprotein concentration is important prognostically in patients with Waldenström macroglobulinemia (WM) [8], but quantification of M-proteins in beta fraction is not often easy because of intersecting non-immunoglobulin protein fractions. Here, we presents a case of atypical migration of monoclonal Ig M in a patient with WM where the M spike has been seen in beta 1 fraction and the difficulties his implies for the quantification of monoclonal protein by electrophoresis when measurement are contaminated by normal serum proteins in this region.

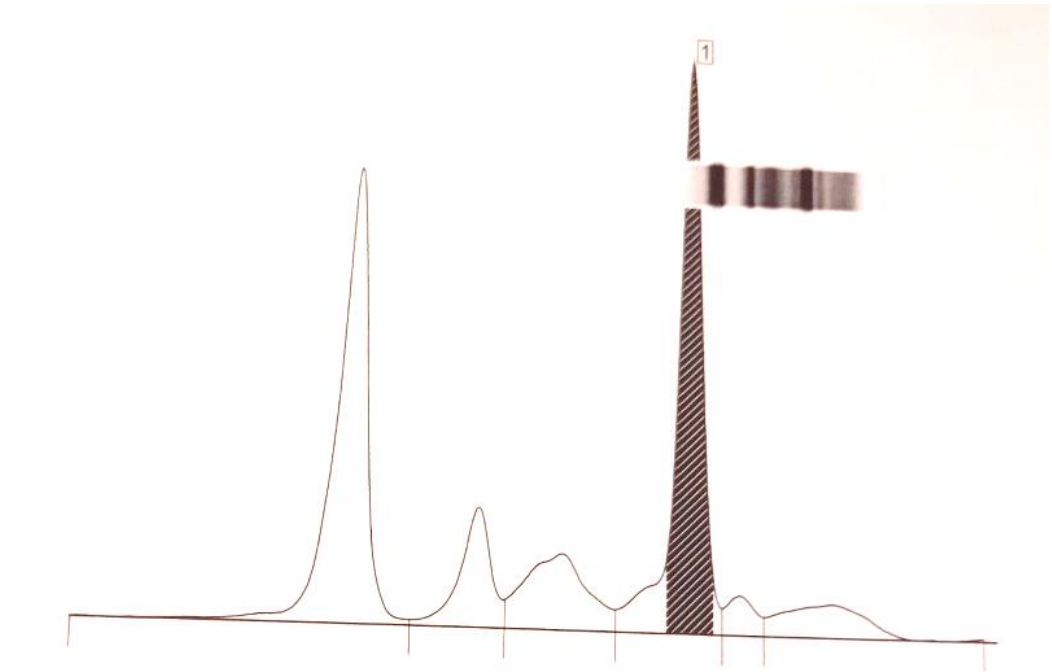
2. Case study

A 67-year-old caucasian man was referred in an hospital center of onco-hematology for fatigue, anemia and perianal lesion. Physical examination showed conjunctival icterus and generalized lymphadenopathy. Computerized Tomography imaging showed splenomegaly in the patient. In the laboratory examinations performed, the complete blood count test showed a normochromic anaemia with leucopaenia (red blood cell count (2.54 × 10^{12}/L), hemoglobin (74 g/L), MCV (88.6 fl), MCH (29.1 pg) and

MCHC (32.9 g/dl), white blood cell count was 0.02×10^9/L and platelet count was 100×10^3/µL. Biochemical investigations revealed normal serum calcium concentration at 2.2 mmol/l (normal reference value NV, 2.2–2.6 mmol/l) , serum creatinine at 43 µmol/l (NV, 69–115 µmol/l), serum total protein was 56.3 g/l and very low level of transferrin at 0.77 g/l (NV, 2–4 g/l). He has normal AST, ALT and LDH levels, total bilirubin was 34.20 µmol/l (NV<17 µmol /l). The level of ferritin was 1874 ng/ml (NV, 18 - 270 ng/ml) and C-reactive protein was 32.58 mg/l (NV<6 mg/l). The bone marrow biopsy showed approximately 47% involvement with lymphoplasmacytic lymphoma cells with expression of CD79a (> 15% of plasma cells), CD20 (> 15% of plasma cells) and CD138 a (> 15% of plasma cells) on immunohistochemistry. The karyotypes of the bone marrow were not available. Capillary serum electrophoresis (Figure1) exhibited a monoclonal peak in the β1 fraction of 18.2 g/l with reversal of albumin-to-globulin ratio and hypogammaglobulinemia at 3.8 g/l, immunofixation showed an IgM kappa monoclonal protein. Urinary free light chains Kappa (Bence Jones proteins) were detected by immunofixation. The presence of lymphoplasmacytic lymphoma (LPL) in the bone marrow and an IgM monoclonal protein with elevated concentration of kappa light chains in the peripheral blood confirmed a diagnosis of WM. Therefore, the patient was referred for further management and treatment. During the hospital stay patient developed neurologic symptoms with peripheral neuropathy and he died few days later.

3. Results and Discussions

Waldenström macroglobulinemia (WM) is a rare B-cell malignancy. The World Health Organization defines WM as infiltration of lymphoplasmacytic lymphoma in the bone marrow with a monoclonal immunoglobulin M (IgM) protein [9,10]. Ig M is structurally distinct from the other immunoglobulin subtypes, it's usually pentameric form with five Ig M monomers, each monomer comprised of two μ heavy chains and two κ or λ light chains, are covalently linked by a polypeptide of 137 amino acids or joining chain (J-chain) [11], the absence of the junction chain generally leads to the aggregation of IgM into hexamers [8]. In humans, the increase in the hexameric form of IgM is present in certain pathologies such as WM [12-14]. On capillary serum protein electrophoresis, the most common area for monoclonal protein migration is the gamma fraction. Monoclonal immunoglobulin is normally visible as a peak (M peak) and measuring absorbance at 210 nm allows for determining its concentration in relation to the total protein concentration of the serum [5]. Waldenstrom and Kunkel later identified that Ig M in patient migrated close to β-globulin using immuno-electrophoresis and ultracentrifugation techniques [15]. Capillary electrophoresis provides a clearer separation of β_1- and β_2-globulins, this separation improves detection of monoclonal proteins and quantified monoclonal peaks more accurate than agarose gel electrophoresis where there are variable affinities of the proteins for the dye [4,16]. In our case, the M spike of Ig M has atypical migration to the beta1 region, the presence of monoclonal proteins in the beta fraction are often of the Ig A [5] or free light chain type. An increase of beta 1 -proteins is seen with iron-deficiency due to high levels of free transferrin or in hemolysis with free hemoglobin [17]. When M-proteins migrate in the β1 region, the presence of transferrin changes the shape of the immunoglobulin peak which is hidden under non-immunoglobulin protein and the patients with iron-deficiency anemia may have a beta1 peak due to increased transferrin concentration, resembling a monoclonal component [12, 17], in this cases, immunofixation should be performed. In our case, the patient has an extra spike of Ig M (18.2 g/l) that is discernible from normal beta proteins with a very low level of transferrin at 0.77 g/l which doesn't interfere with the M protein. After diagnosis, the quantitation of monoclonal protein (M-protein) is crucial for stratifying risk and monitoring patients. The approach which enables estimation of monoclonal fraction is densitometry using the tangent skimming or perpendicular drop methods [13,18], when M spike is found in the gamma globulin fraction. The exact quantification of M-protein in the β region can be masked by transferrin, complement component 3 and β lipoprotein that migrate in this region [5, 14]. Fibrinogen will migrate to the β-γ region on SPE and obscuring the detection of M-protein. Hemolysis, commonly interference results in the presence of pure hemoglobin or as the hemoglobin-haptoglobin complex, both running in the β and α_2 region can confound SPE [5]. There is no standardization of how to handle beta region abnormalities. During treatment, the amount of monoclonal protein changes over time and M spikes present difficulties in quantification using the tangent method due to variability in the measurement of β-migration [19, 20]. For Ig A monoclonal gammopathies migrating in the β-regions, when the monoclonal protein band is obscured by the normal β components, Willrich MA and al [7] advocates splitting the β band as an M peak if the β fraction is >20 g/l (2 g/dl). It's useful in these cases to assist monitoring of IgA and IgM proteins by using quantitative immunoglobulins. Capillary electrophoresis and immunosubtraction is a high resolution electrophoresis which allows a clear separation of transferrin and C3, the measurement of M-spikes with a corrected perpendicular drop guided by results from an immunosubtraction pattern on the same specimen can be accurate and reproducible [7, 21]. Serum immunosubtraction patterns using CZE accurately identifies the area of the β-region that has been subtracted and thus calculates the amount of the normal subjacent constituents, such as transferrin in the β region, and provide a reliable value [20, 22].



Nom	%		Normales %	g/L	Normales g/L
Albumine	33,2	<	55,8 - 66,1	18,7	40,2 - 47,6
Alpha 1	8,8	>	2,9 - 4,9	5,0	2,1 - 3,5
Alpha 2	11,5		7,1 - 11,8	6,5	5,1 - 8,5
Beta 1	37,0	>	4,7 - 7,2	20,8	3,4 - 5,2
Beta 2	2,7	<	3,2 - 6,5	1,5	2,3 - 4,7
Gamma	6,8	<	11,1 - 18,8	3,8	8,0 - 13,5

Rapp. A/G : **0,50** P. T. : **56,3** g/L

Figure 1 : M-spike in the β1 region on capillary serum protein electrophoresis

4. Conclusions

The presence of Ig M monoclonal protein in the beta 1 fraction is unusual on capillary serum protein electrophoresis. We have to be vigilant about the various degrees of asymmetry of M-spike during the interpretation of SPE. The increase in the concentration of certain physiological proteins can generate similar peaks sometimes resembling a monoclonal component. Further, quantification of monoclonal protein and electrophoretic migration position is needed when monitoring patients and can be challenging because of overlapping non-immunoglobulin protein fractions.

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