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Serm Immuno Fixation Electrophoresis as a Diagnostic Method for Monoclonal Gammopathies in Patients with Multiple Myeloma

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Abstract

B cells clonal expansion (producing abnormal amounts of immunoglobulins) reflect conditions causes a group of disorders called monoclonal gammopathies. They may be appearing as a range of diseases that consist of multiple myelomas (MM). The aim of this research is to use quantify and identify monoclonal gammopathy by serum immunofixation electrophoresis (SIFE) beside the serum protein electrophoresis (SPEP) assay as a tumor marker in the diagnosis of suspected multiple myeloma cases. Serum samples were collected from 94 patients with MM, and 30 persons as control, SPEP and SIFE were used for both groups. M band determined and evaluated of M protein by the Hellabio instruments. The results of this study showed significant elevation ($p<0.001$) in the group of patients with MM compared to control as follows: 49 (52.13%) for IgG kappa, 23 (24.46%) for IgG lambda, 10 (10.63%) for IgA kappa, 4 (4.25%) for IgA lambda, 6 (6.38%) for IgM kappa, and 2 (2.12%) for IgG monoclonal gammopathy. SPEP ought to be proposed as the original test for the establishing of doubted cases of multiple myeloma. IFE is the gold mark now and ought to be performed to certify the existence of an M-protein and to diagnostic its light chain and heavy chain isotype, which also leads to increase the sensitivity of diagnosis in suspected multiple myeloma cases by using SIFE beside the SPEP assay and utilized for detection and quantification of monoclonal gammopathy.

Keywords: Multiple myeloma, M component, monoclonal gammopathy, serum immunofixation electrophoresis.

الخلاصة

توسع الخلايا البائية (إنتاج كميات غير طبيعية من الجلوبيولينات المناعية) تعكس الظروف التي تؤدي إلى مجموعة من الاضطرابات التي تسمى اعتلال gammopathies أحادي النسيلة. قد تظهر على أنها مجموعة من الأمراض التي تتكون من الأورام النقوية المتعددة (MM). الهدف من هذا البحث هو استخدام القياس الكمي وتحديد اعتلال غامض وحيدة النسيلة(monoclonal gammopathy) عن طريق الترحيل الكهربائي لمصل المصل (SIFE) بجانب فحص الترحيل الكهربائي البروتين في الدم (SPEP) كمؤشر للورم في تشخيص حالات المايلوما المتعددة المشتبه فيها. تم جمع عينات من المصل من ٩٤ مريضا مع MM ، ٣٠ شخصا كمجموعة السيطرة ، وتم استخدام SPEP و SIFE لكلا المجموعتين. حددت حزمةM ا وتقييم البروتين M بواسطة جهازHellabio. أظهرت نتائج هذه الدراسة ارتفاع معنوي ($p<$٠,٠٠١) في مجموعة المرضى الذين لديهم MM مقارنة بمجموعة السيطرة كما يلي: ٤٩ (٥٢,١٣٪) لـ IgG kappa، 23 (24.46٪) لـ IgG lambda، (10.63٪ 10) لـ IgA kappa، 4 (4.25٪) لـ IgA lambda، 6 (6.38٪) لـ IgM kappa ، و ٢ (٢,١٢٪) لـ IgG monoclonal gammopathy. ينبغي اقتراح SPEP كاختبار أساسي لإنشاء حالات مشكوك فيها من المايلوما المتعددة. IFE هي العلامة الذهبية الآن ويجب أن يتم تنفيذها للتأكد من وجود بروتين M ولتشخيص سلسلته الخفيفة وسلسلة isotype الثقيلة ، مما يؤدي أيضًا إلى زيادة حساسية التشخيص في حالات المايلوما المتعددة المشتبه فيها باستخدام SIFE بجانب اختبار SPEP واستخدمت للكشف عن وتقدير الكمي لاعتلال الأعصاب وحيدة النسيلة.

Introduction

A plasma cell malignancy is called multiple myeloma (MM), also it is familiar as symptomatic plasma cell myeloma. It is distinguished by the bone marrow plasma cells clonal spread that excretes an immunoglobulin free light chain (FLC) or a monoclonal paraprotein[1]. It is called multiple because plasmacytoma is seen at multiple sites[2]. Multiple myeloma is a known lymphoproliferative condition of the plasma cell, when it is untreated a usual median death is30 months[3] or 36-40 months at the best institutions[4][5], and also it remains generally an incurable disease[6]. Multiple myeloma and several diseases including Waldenströmsmacroglobulinemia, amyloidosis, plasmacytoma, monoclonal gammopathy of unevaluated significance, and systemic amyloid light chain, are monoclonal gammopathis[7]. The monoclonal gammopathies are also known as dysproteinemias or paraproteinemias, which are a class of conditions distinguished by the multiplication of single or multiple replicas of differentiated B lymphocytes[8], which can yield an immunologically homogeneous immunoglobulin often, denoted as amonoclonal or paraprotein(M) protein.The serum M-protein can form of an immunoglobulin, the light chain (kappa or lambda type) solitary or hardly the heavy chain only, while the heavy chain is made up of the immunoglobulin classes such as: G, A, M, D or E. Monoclonal gammopathies resulted of the overproduction of a single anomalous clone of a B lymphocyte or plasma cell. The monoclonal immunoglobulin is distinguished as a band of narrow migration on urine or serum electrophoresis (M-component) and when the band constitute is a monoclonal free light chain, it is titled as Bence Jones protein (BJP). In some cases, biclonal or very rarely triclonal may produce[9].The tests for diagnosing and categorizing a monoclonal or a polyclonal gammopathy involves urine, serum protein electrophoresis, and serum, urine immunofixation electrophoresis, and quantifiable serum free light chain (SFLC). In the clinical laboratories for the recognition and proof of identity of paraproteins, serum protein electrophoresis (SPEP) is widely used. While SPEP cannot conclusively identify a M-protein. For that object, electrophoresis with immunochemical ways must be employed both, in order to increase sensitivity for diagnosing plasma cell conditions, peak laboratories nowadays do SPEP with SIFE determinations[10][11].

Methods

This study was conducted in Erbil city/Iraq. During the period from Jun 2014 till November 2015, ninety four blood samples were collected from patients [40 females with mean age ±SD (62 ± 2.7) years and 54 males with mean age ± SD (64 ± 3.8) years] who were selected from newly diagnosed multiple myeloma patients by physicians, which were admitted to Nanakaly Hospital and clinics in Erbil city, while 30 control was collected [13 females with mean age SD (56 ± 5.1) years and 17 males with mean age± SD (60 ± 6.3) years].SPEP was performed on cellulose acetate strips by using a ready-made buffer (pH 8.6). The cellulose acetate strips were initially soaked in the buffer solution and the extra amount of buffer was removed by placing them in between two Whatman no-1 filter papers. Then, the strips were placed on the central compartment of the electrophoresis chamber. Two filter paper strips were placed on both the sides of the cellulose acetate strip to connect them with the two buffer containing chambers on both the sides of the electrophoresis chamber. Then, 10 microliter of the serum samples were loaded on the cellulose acetate strip at the sources of the origin. Then, the electrophoresis chamber was connected to the power pack and it was subjected to electrophoresis. After one hour, the strips were removed and they were stained by using Ponceu S. After destining them by using the reagent which was supplied by the same company, the separated protein fractions could be visualized. The estimation of the individual protein fraction was done by densitiometry. The M band could be detected visually and the concentration of the M protein was estimated automatically by the densitometer.

In immunofixation electrophoresis, proteins are fractionated on electrophoretic strips, but not stained. Each lane is overlaid with monospe-

cific antisera, usually with activity against the three major immunoglobulin classes (IgG, IgA, and IgM), and against free and bound (intact) k and l light chains. Immunoglobulins are precipitated by the antisera in the gel. After a few hours, the gels are washed to remove unprecipitated proteins and then stained. If a M-component is present, it appears as a band coincident with the paraprotein. It can be characterized as IgG, IgM, IgA, and k or l, depending on the pattern of precipitation. The serum was collected and each one was quantified and classified to their iso types by using Hellabio protein electrophoresis kits and Hellabio immunofixation kit with Hellabio and Hellabio scan gel analyzer instruments. Whole statistical analysis was done with the Statistical Package for the Social Sciences (SPSS) 15.0 Package (SPSS Inc., Chicago, IL, USA). Expressive statistics were existed as arithmetic mean ±standard deviation and percentages.

Results and Discussion

The results of SPEP for the patients with multiple myeloma and healthy subjects were shown in Figures 1, 2 and Table1, respectively. Figure 3 showed the results of SIFE for 94 patients with multiple myeloma, which is categorized into different categories depending on their immunoglobulin light and heavy chains as follows: 49 (52.13%) were for IgG kappa, 23 (24.46%) for IgG lambda, while 10 (10.63%) for IgA kappa, 4 (4.25%) for IgA lambda, 6 (6.38%) for IgM kappa, and 2 (2.12%) for IgG ,the results were significantly elevated ($p<0.001$) for the patients. In another site, 30 healthy individuals were participated in the current study. Figures 4 and 5 represents SIFE for the patients with MM type IgG kappa and healthy individuals respectively.

Table 1: Serum protein electrophoresis for a sample with MM monoclonal IgG lambda.

Fraction	Value (%) MM	Control (%) Range
Alb.	37.15	4.4-5.7
Alpha1-	4.29	1.6-3.1
Alpha2-	7.77	4.3-7.4
Beta-	9.16	6.1-11.5
Gamma-Glob.	41.05	6.9-15.8

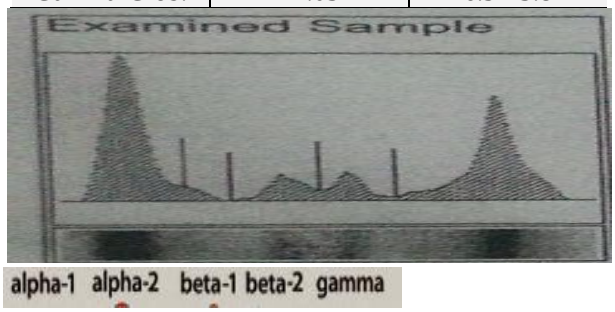

Figure 1: serum protein electrophoresis for patients with MM.

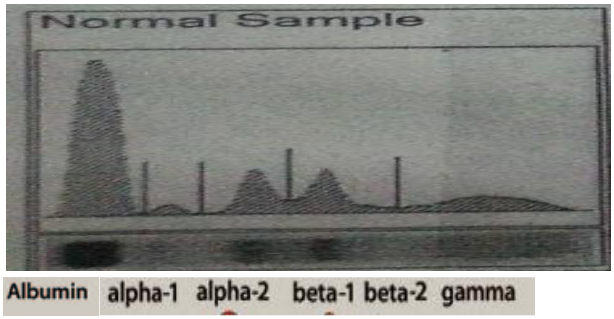

Figure 2: serum protein electrophoresis for healthy subjects.

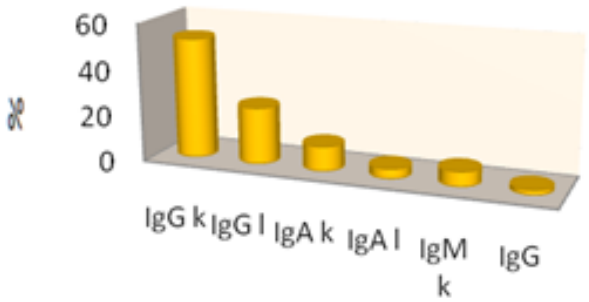

Figure 3: The serum Immunofixation Electrophoresis results in patients with MM**.**

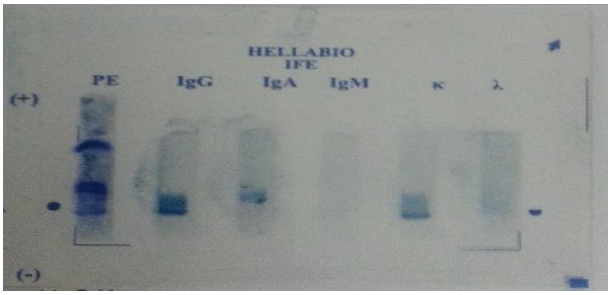

Figure 4: serum immunofixation electrophoresis in patients with multiple myeloma.

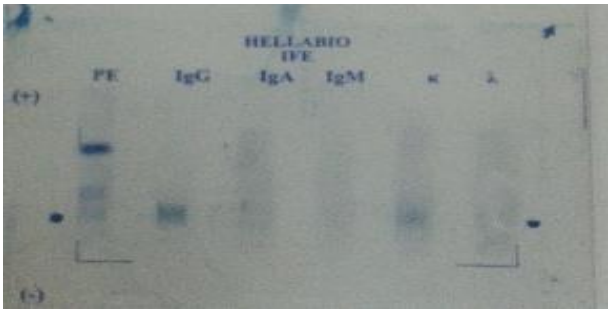

Figure 5: Serum Immunofixation Electrophoresis for healthy subjects**.**

In the clarification of SPEP, more attentiveness is pointed to the gamma area, which is largely consisting of immunoglobulin[12].Various disorders can be due to a raise in the gamma area, but the one with a homogenous point in the gamma globulin area had unusual attention[13]. Monoclonal gammopathies are results of the multiplying of alone, normally malignant clone of plasma cells, which result from of alone class of immunoglobulins[14], heavy or light chains or both. These proteins are known as M (monoclonal) or paraproteins, when the serum performing on electrophoresis and the M component or the M protein is diagnosed as a sharp symmetric point (M spike) with α2, β, or γ movability. The most common cause of paraproteinaemia is MM[12][13], which is a tumor indicator specific for monoclonal gammopathies case the clonal creation of immunoglobulin[15]. The detection of MM was done by different measurements including the following: low levels of Hb (anemia), existing of a monoclonal M protein in the urine or plasma,[9], hypercalcemia[16], and producing skeletal demolition that causes in bone pain and fracture[17], recurrent bacterial infections, infiltration of plasma cells in the bone marrow, and etc.[18]. Several quantities of urine and serum for the M-protein are also utilized to describe relation of treatment and follow the progression of the sickness[9].

The detailed results of the SPEP for the patients are outlined in Figures 1, 2 and Table 1, which is shown the decreasing of albumin level in the percentage of the serum proteins within huge increasing in γ-globulin spike. Such a huge raise in γ-globulin is due to the observation of MM. The huge peak of γ-globulin area is sharp relatively. These results were agree with the results of O'connell *et al.*[19] they` found increase amount of γ-globulin[20] and decreasing albumin levels in multiple myeloma patients[12][19]. M-protein is commonly seen as a restricted band which is frequently observed on γ or β globulin district, also it may be observed on α2 globulin range but this is as very unusual situation[12].

The SPEP is limited at low monoclonal immunoglobulins levels[21], so in order to increase the sensitivity by utilizing of SIFE for diagnosing of the monoclonal immunoglobulins, and to categorize the light or heavy chain isotype, so it should be utilized SPEP and SIFE tests together for the identification of suspected multiple myeloma [13][22], and to attain additional specific information about the kind of abnormal antibodies present[23]. Novel antibodies have been designed (heavy/light chain antibodies), which distinctly recognize the different light chain kinds of every immunoglobulin group, that is, IgGκ, IgGλ, IgAκ, IgAλ, IgMκ, and IgMλ. They provide precise amount of the involved and uninvolved immunoglobulin of affected isotype of the patients [20]. A large amount of myelomas make complete heavy chain immunoglobulin molecules of about IgG (55%), IgA(22%)[24], Bi-clonal (2%), light chain (16%), Bi-clonal (2%), and IgM (0.5%), while IgD and IgE are uncommon[25]. The amount of paraprotein made is frequently proportional to the mass of the tumor. The extreme Ig fragments (light chains or parts of heavy chains) amounts are also made in approximately 85% of cases. In about 10-20% of multiple myeloma patients' dimers of light chains, either of the κ or λ type (Bence Jones proteins). In 75% of cases, paraproteins were existed in both urine and serum[23]. The detailed results of SIFE were summarized in Figures 3, 4 and 5, while Figure 3 shows the monoclonal gammopathy results for patients with Multiple Myeloma which significantly increase ($p<0.001$) as follows, 10 (10.63%) for IgA kappa, and 4(4.25%) for IgA lambda in ratio about 3:1 as similar to the ratio of results by Boyle et al study[26]. It is implicated that irregular ratios of k/l free light chain only causes as of plasma cell or clonal B-

lymphoid proliferative condition, and is construed as a proof of serum monoclonal light chains[8]. The abnormal cell (s) may create an intact immunoglobulin, which is rarely contain only heavy chains[2] as agree with this study within 2 (2.12%) of IgG cases, within 6 (6.38%) were in IgM kappa in this study, while the SIFE for anodal and cathodal boundaries are reliable with IgG k M proteins, which is the most notable result 49 (52.13%) for IgG kappa which elevated significantly ($p<0.001$)in this study as seen in Figure 3, in another site the SIFE reveals that the limitation in the transferrin-range was because of the smaller cases 23 (24.46%) for IgG lambda which elevated significantly ($p<0.001$) monoclonal gammopathy comparing with IgG k M proteins and these results are agreed with Attaelmannan*et al* [2], with ratio of about 2:1for k/l in IgG, and this is similar to the results of Kyle[17]. which is established that the kappa light chain was twice as recurrent as lambda (in the serum they consistent with the normal 2:1 ratio of k/l) in MM cases[27]. In MM, IgG is the most common immunoglobulin appeared and increased, while IgA occurs in the second most common one, as similar to the results of this study. If a k or l M-component is observed in serum in the lack of the IgG, IgA, or IgM heavy chain, it is necessary to assay the sample for IgD and IgE [2]. Comparatively new serum assays for free kappa and lambda light chains of immunoglobulins reveal the results of free light chains is more precise than urine examinations[28].

Conclusions

SPEP ought to be proposed as the original test for the diagnosing of doubted multiple myeloma patients, by the qualification and quantification of paraproteinemia in the serum. IFE is the gold rating now and ought to be done to ensure the existence of an M-protein and to diagnosis its heavy chain and light chain isotype, which also leads to increase sensitivity of diagnosis in suspected multiple myeloma cases by using SIFE beside the SPEP assay and utilized for detection and quantification of monoclonal gammopathy, interpreted with associating the clinical features clinical features and the bone marrow biopsy.

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