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ORIGINAL ARTICLE

Concordance of Capillary Electrophoresis and Conventional Gel Electrophoresis in Two Different Groups of Patients with Newly Diagnosed vs. Pre-Existing Monoclonal Gammopathy

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SUMMARY

Background: **Serum and urinary protein electrophoresis play an important role in the identification of monoclonal gammopathy. Recently, capillary electrophoresis (CE) has been adapted in many clinical laboratories because of several advantages such as short turnaround time, automation, and high reproducibility. However, there have been unsolved concerns for the concordance between conventional gel and automated capillary electrophoresis methods for protein separation in clinical specimens. In this study, we investigated the diagnostic performance of both methods for detecting monoclonal (M) protein.**

Methods: **From February 2012 to August 2015, a total of 3,013 CE tests were performed in our hospital. Among these cases, we reconfirmed results of CE (Capillary 2, Sebia, Lysse, France) with those of conventional agarose gel electrophoresis (GE) (Hydragel 4IF, Sebia, Lisses, France) in 28 specimens from 24 patients with newly diagnosed monoclonal gammopathy (group 1). In addition, 22 cases from 15 patients with previously diagnosed monoclonal gammopathy presenting indeterminate or suspicious results on CE (group 2) were also reconfirmed with GE.**

Results: **We compared the results between the two electrophoresis methods in two different groups of patients with newly diagnosed discrete monoclonal peaks vs. pre-existing monoclonal gammopathy with obscure results in follow-up courses. In group 1, agreement rate was 100% (28/28) and there was no discrepant result between these two electrophoresis methods. In contrast, group 2 showed 86.4% (19/22) agreement rate and 0.67 Cohen's kappa value (95% confidence interval, 0.51 - 1.02).**

Conclusions: **According to our results, both electrophoresis methods can be used with the same level of assurance at the time of initial diagnosis for monoclonal gammopathy. However, in patients with previously diagnosed monoclonal gammopathy in follow-up course after appropriate treatments, discordant results can be observed due to the reduced amount of M proteins. Therefore, we suggest that some ambiguous cases with very small amounts of M components require a combination of both CE and GE methods for accurate interpretation to confirm the presence of M proteins.**

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INTRODUCTION

Monoclonal gammopathy is characterized by proliferation of plasma cells from single clones which produce homogenous monoclonal (M) proteins [1]. Measurement of M protein is critical for diagnosing and monitoring monoclonal gammopathy [2]. Serum and urinary protein electrophoresis (PEP) have been widely used and play an important role in the detection of M protein for diagnosis and follow-up of monoclonal gammopathy such as multiple myeloma (MM), Waldenström macroglobulinemia, and other plasma cell disorders [3, 4]. Once monoclonal gammopathy is proven by PEP, MM should be differentiated from other monoclonal gammopathies, and the rest of patients need to be closely monitored because of the possibility of malignant progression to MM [1]. Agarose gel electrophoresis (GE) has been recognized as a common clinical method for routine PEP for a long time [5]. Capillary electrophoresis (CE) was developed by Mikkers et al. in 1979, and it appeared as a new technique for protein separation in clinical specimens [6]. At present, CE is generally accepted as a suitable methodology for separation and characterization of proteins in serum and urine specimens [7,8]. Although GE is still the method of choice, CE has been increasingly adopted in many clinical laboratories for routine PEP tests, because it has several advantages over the conventional method such as speed, convenience, and high reproducibility [9-11]. Although a few previous studies compared the results between CE and GE, there have been unresolved concerns regarding the concordance between these 2 electrophoresis methods in clinical specimens for identifying monoclonal gammopathy [5,12]. In this study, we investigated the diagnostic performance of both methods by assessing the concordance rate for detecting M protein in two different groups of patients with newly diagnosed vs. pre-existing monoclonal gammopathy.

MATERIALS AND METHODS

From February 2012 to August 2015, a total of 3,013 CE tests were ordered and performed in our hospital. Among these cases, we reconfirmed CE results with conventional GE in 28 specimens from 24 patients with newly diagnosed monoclonal gammopathy (group 1) with the same specimens. In addition, 22 cases from 15 patients with previously diagnosed monoclonal gammopathy presenting indeterminate or suspicious results on CE (group 2) were also reconfirmed with GE. These 50 cases were comprised of 40 serum specimens and 10 urine samples. Clinical data were gathered through medical chart review in these 50 cases and were compared in both CE and GE. A part of this patients group was partially included in our previous studies [13-17]. CE and subtraction immunotyping electrophoresis (IT) were done using Capillary 2 (Sebia, Lysse, France) according to the manufacturer's directions. GE and immunofixation electrophoresis (IFE) were performed on Hydragel 15 HR gels (Sebia, Lysse, France). Routine chemistry tests were performed on a Toshiba chemical analyzer (Toshiba, Nasushiobara, Japan). Free light chain (FLC) assays were performed by a BN II analyzer (Siemens, Marburg, Germany) using Freelite™ Human Kappa and Lambda kits (The Binding Site Ltd., Birmingham, UK).

The concordance rate between CE and GE was assessed by calculating Cohen's kappa value. Calculations were performed using the SPSS software (v.22; SPSS Inc., Chicago, IL, USA) and Excel 2007 (Microsoft, Redmond, WA, USA). p-values < 0.05 were considered statistically significant. Institutional Review Board (IRB) approval was obtained for this study (IRB approval number (2015-06-111-007).

RESULTS

Table 1 presents comparative data for the identification of M proteins between GE and CE in 50 cases. We compared results between CE and GE methods in two different groups of patients with newly diagnosed discrete monoclonal peaks vs. pre-existing monoclonal gammopathy with obscure results in follow-up courses. Group 1 was comprised of 28 cases from 24 patients with newly diagnosed monoclonal gammopathy, agreement rate was 100% (28/28) and there was no discrepant result between CE and GE methods for identification of M proteins and classification of Ig isotype. In contrast, group 2 including 22 cases from 15 patients with previously diagnosed monoclonal gammopathy revealed 86.4% (19/22) in agreement rate and 0.67 in Cohen’s kappa (95% confidence interval, 0.51 - 1.02). Only three cases in group 2 presented discordant results between these two methods. Of them, the first case with a very small M peak with free lambda type was observed only by CE but was not evident by GE (Figure 1A). Conversely, in the second case, GE characterized IgA and kappa type M proteins, while CE did not yield any discrete M peak (Figure 1B). The third case, which showed an IgG M peak by CE, was identified as oligoclonal bands by GE without any definite evidence of M components (Figure 1C).

In these three cases with discrepant results between CE and GE, the medical records were thoroughly reviewed to assess clinical information and other biochemical indicators (Table 2). In patient 1 with multiple masses in the thoracic area, plasma cell tumor was diagnosed by biopsy in 2008. However, there was no distinct plasma cell proliferation in bone marrow (BM) examination. The M peak detected at the initial PEP test disappeared

Table 1. Identification results of monoclonal immunoglobulins by capillary electrophoresis compared with gel electrophoresis.

Agarose gel electrophoresis	Capillary electrophoresis							
	IgG, κ	IgG, λ	IgA, κ	Free κ	Free λ	OCB	Other †	ND
IgG, κ	14	-	-	-	-	-	-	-
IgG, λ	-	6	-	-	-	-	-	-
IgA, κ	-	-	8	-	-	-	-	1**
Free κ	-	-	-	7	-	-	-	-
Free λ	-	-	-	-	6	-	-	-
OCB	-	-	-	-	-	1	1***	-
Other	-	-	-	-	-	-	-	-
ND	-	-	-	-	1*	-	-	5

* Lambda type M peak was observed by CE, while M protein was not detected by GE, ** M peak was not observed by CE, while IgA and kappa type M protein was detected by GE, *** M peak was observed by CE and oligoclonal bands were detected by GE, † IgG M peak was observed in CE. Abbreviations: CE - capillary electrophoresis, GE - agarose gel electrophoresis, OCB - oligoclonal bands, ND - not detected.

Table 2. Clinical and laboratory information in 3 discrepant cases between capillary electrophoresis and gel electrophoresis.

	Monoclonal immunoglobulins		Creatinine, μmol/L (44.2 - 88.4)	Calcium, mmol/L (2.1 - 2.55)	Hemoglobin, mmol/L (7.45 - 9.93)	Serum FLC ratio	Radiologic finding *	Marrow plasma cells, % *
	CE	GE						
Patient #1	Free λ	ND	53.04	2.33	7.57	0.667	Suggestive of plasmacytoma	1.0
Patient #2	ND	IgA, κ	61.88	2.58	7.45	329.011	Severe osteopenia	23.2
Patient #3	IgG	OCB	70.72	1.95	6.14	1.191	Severe osteopenia	27.0

* Radiologic finding and marrow plasma cells % were the results at the time of initial diagnosis.
Abbreviations: CE - capillary electrophoresis, GE - agarose gel electrophoresis, OCB - oligoclonal bands, ND - not detected.

after receiving treatment; however, the small peak reappeared in CE during the follow-up course. Patient 2 initially showed increased kappa:lambda FLC ratio of 329.011 with mild hypercalcemia, and 23.2% of plasma cell proliferation was confirmed by BM examination in 2014. PEP test was not ordered at the time of initial diagnosis through BM; however, the PEP result 3 months after the therapeutic course revealed the presence of a very small M peak in GE. Patient 3 was previously diagnosed with MM in 2009 with 27% of plasma cells in BM examination. Serial PEP tests were performed since the initial diagnosis, and the M peak reappeared in PEP results in CE after 3 years of remission period.

DISCUSSION

The purpose of this study is to compare the diagnostic performance between two electrophoresis methods to detect and identify M proteins. To the best of our knowledge, comparison data of CE and GE among clinical specimens has been rarely reported in previous studies. Because there has been no assurance that CE can be analytically sufficient to replace traditional GE, clinicians seem to have doubted whether CE can be used as a reliable alternative method for identifying M components instead of the conventional and gold standard method of GE in all clinical conditions. Several previous studies demonstrated that CE was slightly more sensitive than GE for detecting the M peak in terms of its prevention of application artifacts and greater detection capability of M proteins in the beta-region [4,5,10,18]. On the other hand, the methodological difference of CE appears to result in relatively lower specificity than GE, for example, interference of contrast substances [5,11]. However, the accumulated data seem to not be sufficient for providing confirmation that these two electrophoresis methods yield the same results all the time.

In this study, conventional GE and CE were perfectly

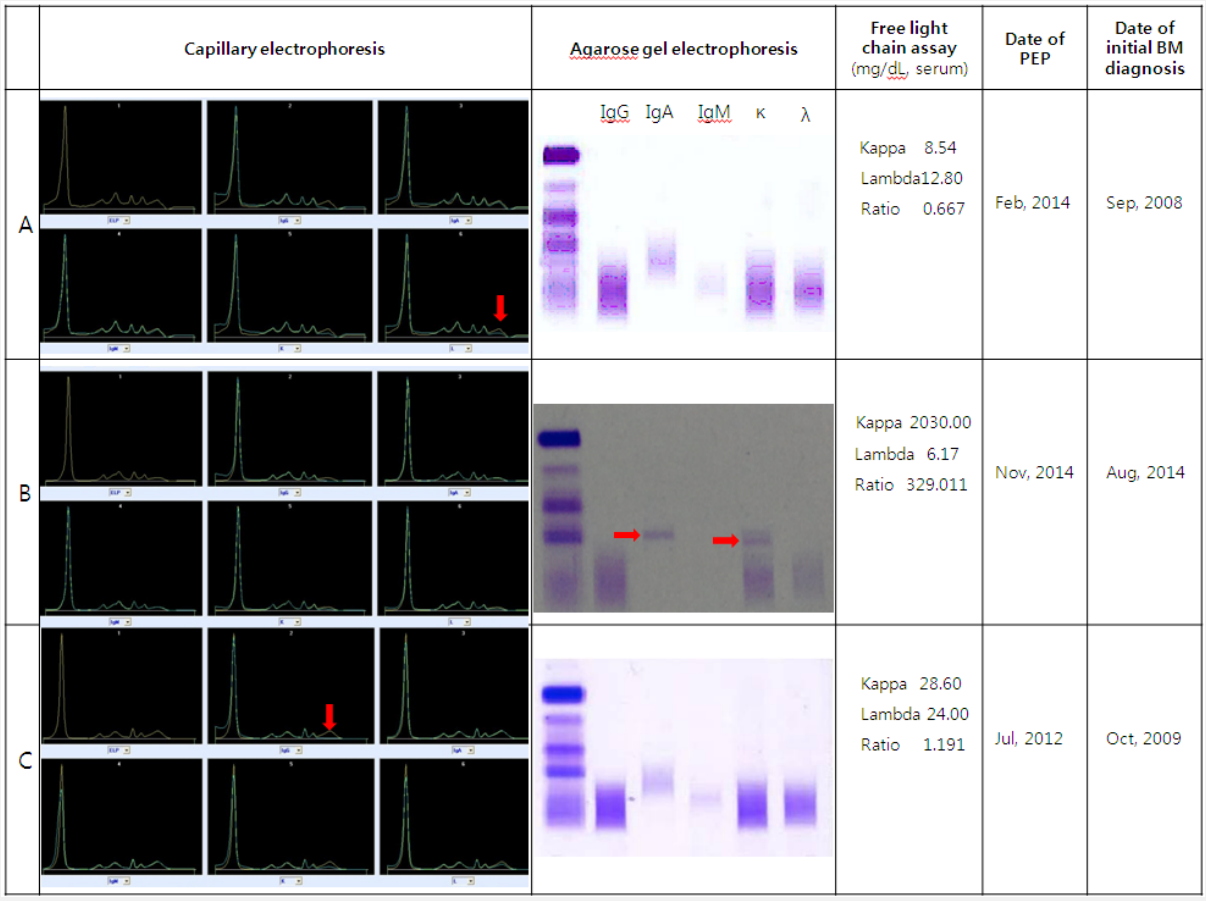

Figure 1. Serum electrophoretic patterns obtained by capillary electrophoresis and agarose gel electrophoresis in three discordant characteristics.

Vertical arrows show M peaks in gamma region on CE and horizontal arrows indicate M components on GE. In patient 1, a very small free lambda type of M peak was shown in CE, while M protein was not evident by GE (A). In patient 2, IgA and kappa type M proteins were clearly characterized by GE, whereas any M components were not observed by CE (B). In patient 3, IgG M peak was observed by CE, while GE confirmed it as oligoclonal bands (C). Abbreviations: CE - capillary electrophoresis, GE - agarose gel electrophoresis, BM - bone marrow.

concordant in newly diagnosed patients with definite M peaks without any discrepancy of the isotype of M proteins. This finding seemed to result from the presence of a sufficient amount of M proteins in newly diagnosed patients with a prominent M peak. In cases of new patients showing a marked size of M peak, both CE and GE method seem to be equally appropriate for initial diagnosis. All three discrepant cases occurred when the size of M peak was decreased in patients who had previously been treated for monoclonal gammopathy. Therefore, clinical judgment might be difficult owing to discordant results in follow-up patients whose M components have been significantly decreased. Moreover, accompanied polyclonal gammopathy or oligoclonal gammopathy can mask small sized M peaks into the background in gamma region on only one of these two methods, according to patient 3 in this study [16,17,19]. For distinguishing monoclonal components from a small amount of polyclonal materials, performing surrogate laboratory tests, such as FLC measurement and FLC ratio can be useful. Except for the cases discussed in this study, several troublesome cases may occur. For instance, an IgA peak may occasionally be observed in the beta-2 region. The peak in the beta-region can be identified as a unique symmetrical immunosubtraction pattern. In addition, using PEP and IFE tests by CE and GE methods and careful interpretation of results should be accompanied if there are two peaks present that overlap. In the case of analyzing urine specimens, samples must be concentrated up to 100 times before analysis,

since total protein concentration is relatively low in urine [20]. Because this additional process can enrich concentrations of M proteins, we found no discordant characterization in 10 urine samples analyzed in this study. Ultimately, combination of both CE and GE methods and careful interpretation for the presence of a very small peak are necessary to confirm the presence of M proteins for such ambiguous cases that can happen, especially near remission or recurrence stages of monoclonal gammopathy.

Automated CE, a quick and simple test method with high resolution, is suitable for screening purposes [21]. However, interpretation of IT in CE may require exceptional expertise and be restricted to particular laboratories. Thus, in order to make correct reporting on CE results, complementary use of GE is required according to circumstances. As shown by our results, both electrophoresis methods can be applied with the same level of assurance at the time of initial diagnosis. However, as patients receive proper treatment during monitoring period, the amount of M proteins decreases and the size of peak is also reduced. In this condition, we identify that CE and GE occasionally can make discordant results in follow-up patients with a small sized peak. Therefore, we suggest that some ambiguous cases with very small amounts of M components require a combination of both CE and GE methods for accurate interpretation to confirm the presence of M proteins.

CONCLUSION

Conventional GE and CE results were completely matched and no inconsistent results of isotyping M proteins were observed in patients with prominent M peaks that have sufficient amounts of M protein, such as newly diagnosed patients. However, relatively lower agreement rate was noticed in follow-up patients. Therefore, if clinicians require information to determine remission or recurrence of very small M peaks, combination of both CE and GE methods are highly recommended for accurate interpretation to confirm the presence of M proteins.

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Declaration of Interest:
The authors have no conflicts of interest regarding the publication of this article.

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