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Original Article

Evaluation of Diff Quik Staining Method for Detection of Mono Sodium Urate Crystals in Synovial Fluid Using Light and Polarizing Microscopy: A Cross-Sectional Study

Ina Rahim[1], Jamal Pasha Chowdhury[2]

[1]Senior Consultant (Pathology), Sheikh Russel Gastro Liver Institute, Dhaka.
[2]Classified Specialist in Pathology, CMH, Rangpur.

Abstract

Gout is one of the oldest diseases of the medical history that results from the deposition of monosodium urate crystals in joint structures and in periarticular sites in the form of tophi. The evaluation of synovial fluid is integral for the diagnosis of gout and other arthritis of microcrystals. The objective of this cross-sectional study was to evaluate the analysis of synovial fluid by polarizing and light microscopy using wet film and Diff Quik stained films and evaluating usefulness of Diff Quik stain in identifying monosodium urate (MSU) crystals on permanent mounted slides. It was conducted on 100 clinically suspected gout patients in the Department of Clinical Pathology, Bangabandhu Sheikh Mujib Medical University, Dhaka from May 2008 to April 2009. Polarizing Microscopy detected the presence of MSU crystals in 35.71% cases from wet films and 36.73% cases from Diff Quik stained films. Light Microscopy detected crystals in 28 (28.57%) cases from Diff Quik stained samples and in 31.63% cases from wet film samples. Considering wet film polarizing microscopy as gold standard, the sensitivity and specificity of wet film light microscopy was 88.6% and 100.0% respectively, whereas sensitivity and specificity were 80.0% and 100.0% respectively in Diff Quik light microscopy.In Diff Quik polarizing microscopy, sensitivity and specificity were100.0% and 98.4% respectively. The sensitivity and specificity was highest in Diff Quik stained films examined by polarizing microscopy

Key words: Polarizing microscopy, Diff Quik Staining

Introduction

Gout is recognized as one of the oldest diseases of the medical history affecting many patients worldwide. Commonly the term 'gout' implies 'urate gout', and an inflammatory response to crystal deposition in joints occurs referred to as 'gouty arthritis'. The chronic disease results from the deposition of monosodium urate (MSU) crystals in joint structures and in periarticular sites in the form of tophi when there is an increased urate concentration in blood.

In clinical practice, gout is frequently diagnosed on the basis of hyperuricaemia and other clinical grounds.[1] But Shojania in his study claimed that gout will ever develop in less than 1 in 3 people with hyperuricaemia and in 10% patients with acute attack, serum uric acid levels are normal.[2] The evaluation of synovial fluid (SF) is integral for the diagnosis of gout and other crystal related arthropathies. The gold standard for gout diagnosis is confirmation of MSU crystals by polarizing microscopy (PM) of Synovial Fluid (SF) or tophaceous material. MSU crystals appear as negatively bi-refringent needle-shaped crystals, 120 μm in length.[3]

Examination of crystals in SF by PM was introduced in clinical practice by Hollander and McCarty in 1961, since then a large number of techniques were introduced to identify crystals.[4] Among them, laser microscopy, atomic force microscopy, electron microscopy, energy-dispersive elemental analysis and x-ray diffraction methods provided a definitive measure for diagnosing crystals.[5,6] These complex methods are used in limited centers, mostly in developed countries and usually for research purposes. On the contrary, compensated PM is a relatively simple, affordable technique with a reasonable degree of sensitivity and specificity. It makes crystals to be visualized easily by observing their different shapes and birefringence (splitting a ray of light into two, separated by wide margin) property. Observation of fresh SF sample with polarized filters, but without the compensator,

Correspondence:
Jamal Pasha Chowdhury
Classified Specialist in Pathology
Combined Military Hospital, Rangpur.
E-mail: jpashac@gmail.com

allows the strongly bi-refringent needle shaped MSU crystals to be seen very easily. The use of first-order red compensator is inserted in order to determine the sign of birefringence which helps to differentiate MSU from calcium pyrophosphate dehydrate (CPPD) and other types of crystals. Morphologically, MSU crystals are always acicular (needle shaped), CPPD crystals are acicular, rhomboidal or plate like.[7] So, wet film (WF) compensated PM plays a fundamental role in evaluating crystal deposition diseases with a high degree of certainty and it has been attributed to be a gold standard for diagnosis of gout and related arthropathies.[3,7,8,9] It is a valuable essential bedside procedure in daily clinical practice, but the fluid needs to be examined within a few hours from joint aspiration and the quality of sample deteriorates with time. Also the cytologic examination is difficult in WF.

In 2001, 'Diff Quik' (DQ) staining method for identifying crystals was described by Selvi et al.[9] It is a commercial Romanowsky stain variant used to rapidly stain and differentiate a variety of pathology specimens, a simplified alternative of Papanicolaou stain used in cytological examination. It is a three-step procedure that takes about 20-30 seconds to perform. Cytologic advantages include the large size of cells that withstand shrinkage from immediate fixation and support easier identification of cytoplasmic granularity, inclusions and crystals.[10] DQ stained smears, could provide a useful tool for delayed SF analysis suitable for quality controls, including cytological examination and crystals detection and identification. The overall sensitivity and specificity of DQ stain for crystal confirmation are 94.4% and 87.5% respectively.[9]

The purpose of the study was to evaluate the usefulness of DiffQuik(DQ) staining method in identifying MSU crystals in SF by DQ stained smears by Light Microscopy (LM) and PM and comparing it with the conventional WF preparation by LM and PM, with an aim to assess and compare the definite value of them for diagnosis of crystal related arthropathies.

Materials and Methods

A total of 100 clinically suspected patients were enrolled in the study following inclusion criteria (postmenopausal female, male above 18 years, with clinical features suggestive of gout having mono and polyarthritis). The study was done in the Department of Clinical Pathology, Bangabandhu Sheikh Mujib Medical University (BSMMU) Dhaka with enrolled patients from Internal Medicine, Rheumatology, Physical Medicine and Orthopedics Departments, BSMMU during May 2008 to April 2009. Freshly aspirated SF samples (maximum 5 ml, minimum 0.1ml from smaller joints) from knee, ankle & other joints were examined in the laboratory.

For WF preparations, SF was mixed thoroughly and one drop was placed on a clean slide and a cover slip placed over it. The film was scanned at first under 100 magnification, then at 400 magnification by LM. Presence of crystals was looked for and if present, their shapes were noted.[10] Fluids were then examined using a PM. At first the slide was scanned at low power i.e.100 magnification and looked for the birefringent crystals. If crystals were found, they were focused on high power i.e. 400 magnification to identify the type of crystals.[7]

Then DQ stained slides of SF were prepared according to manufacturer's instruction and examined by LM & PM and the characteristics of crystals were noted.

WF PM examination of SF was considered as gold standard for crystal identification.[8] The results were presented in tables and figures. Validity test, chi-square test and unpaired t-test, were performed. Level of significance was expressed as 'p' value. P value of <0.05 was considered as significant.

Results

Out of total 100 cases, two patients had CPPD crystals, which were excluded and therefore 98 disease cases were considered. Out of the 98 cases, PM detected the presence of MSU crystals in 35 cases (35.71%) from WF and 36 cases (36.73%) from DQ stained samples (Table I). LM detected crystals in 28 (28.57%) cases from DQ stained samples and in 31 (31.63%) cases from WF samples. (Table II)

Table-I: Detection of crystals by polarizing microcopy. (n=98)

	Wet Film (n=98)	Diff Quik Film (n=98)
Crystals detected	35 (35.71%)	36(36.73%)
Crystals not detected	63(64.29%)	62(63.26%)
Total	98(100%)	98 (100%)

Table-II: Detection of crystals by light microscopy. (n=98)

	Wet Film (n=98)	Diff Quik Film (n=98)
Crystals detected	31 (31.63%)	28(28.57%)
Crystals not detected	67(69.37%)	70(71.43%)
Total	98(100%)	98 (100%)

The overall validity of WF and DQ films by light and polarizing microscopy for MSU crystals detection was confirmed by calculating sensitivity, specificity, accuracy, positive and negative predictive values by using the standard statistical methods. (Table III).

Table-III: Sensitivity, specificity, accuracy and positive and negative predictive values of the WF and DQ light microscopy and polarizing microscopy for identification of MSU crystals (n=98).

Validity test	WF LM	DQ LM	DQ PM
Sensitivity	88.6	80.0	100.0
Specificity	100.0	100.0	98.4
Accuracy	95.9	92.9	99.0
PPV	100.0	100.0	97.2
NPV	94.0	90.0	100.0

In WF LM 31 (31.63 %) cases were MSU crystal positive and 67 (68.4%) cases were negative. (Table I) When compared to WF PM (gold standard) among these 31 positive cases, all 31 cases were found positive and no case was found negative in WF (PM) examination. Whereas, in 67 negative cases detected by WF LM, 4 cases were found positive and 63 cases were negative by WFPM. (Table I, II)

In DQ (LM) out of the 98 cases, true positive 28 and no false positive, false negative 7 and true negative 63 cases and In WF (LM) out of the 98 cases, true positive 31 and no false positive, false negative 4 and true negative 63 cases.

Similarly, in DQ (PM) for evaluation of MSU crystal out of the 98 cases, true positive 35 and 1 false positive, no false negative and true negative 62 cases

Considering WF PM as gold standard in DQ LM examination sensitivity, specificity, accuracy, positive and negative predictive values were 80.0%, 100.0%, 92.9%, 100.0%, 90.0% respectively; whereas in WF LM examination it was 88.6%, 100.0%, 95.9%, 100.0%, 94.0% respectively in WF LM. (Table III).

Similarly in DQ PM sensitivity was 100.0%, specificity 98.4%, accuracy 99.0%, positive predictive values 97.2% and negative predictive values 100.0% (Table III).

The amounts of crystals in SF were estimated by a semi-quantitative evaluation described by Linthoudtet al.[11] (Table IV and V)

Table-IV: Estimation of crystals in light and polarized microscopy (Wet Film)

Wet film	Light Microscopy		Polarizing Microscopy	
	n	%	n	%
+	7	20.0	9	25.7
++	9	25.7	7	20.0
+++	15	42.9	19	54.3
Not found	4	11.4	0	0.0
Total	**35**	**100.0**	**35**	**100.0**

Table-V: Estimation of crystals in light and Polarized Microscopy (Diff Quik stained film)

Diff Quik	Light Microscopy		Polarizing Microscopy	
	n	%	n	%
+	13	37.1	6	17.1
++	5	14.3	7	20.0
+++	10	28.6	22	62.9
Not found	7	20.0	0	0.0
Total	**35**	**100.0**	**35**	**100.0**

3+ = at least one crystal on each high-power field (HPF);
2+ = one crystal on every other HPF;
1+ = one crystal or less than one on every other HPF.[11]

Discussion

In 1961, McCarty and Hollander[4] introduced PM for diagnosing MSU crystals in WF of SF and the technique became established as a reliable way of diagnosing gout and other crystal related arthropathies. Moreover, E. Selvi used DQ stain and found the sensitivity and specificity for crystal confirmation was 94.4% and 87.5% respectively.[9] In the current study both the procedures were examined and the result of DQ staining procedure was compared with WF Light and Polarized Microscopy.

In the current study 36 (36.7%) cases were identified as gout as detected by all four methods. Out of these, WF LM identified 31 (31.63%) cases, DQ LM 28 (28.57%) and WF PM 35 (35.71%) cases. Maximum cases were identified by DQ PM which was 36 (36.73%).

In this study, DQLM was used as diagnostic modality for detection of gout; the sensitivity was 80.0%, specificity 100.0%, accuracy 92.9%, (positive predictive value) PPV 100.0% and NPV (negative predictive value) 90.0%. Whereas Sensitivity, specificity, accuracy, PPV and NPV values were 88.6%, 100.0%, 95.9%, 100.0%, 94.0% respectively in WF LM. Similarly, the DQ PM sensitivity was 100.0%, specificity 98.4%, accuracy 99.0%, PPV 97.2% and NPV 100.0% for detecting gout. Selvi E et al[9] had shown that DQ offered 100 percent sensitivity, specificity, accuracy, PPV and NPV in predicting MSU crystals, where the investigators identified all the MSU crystals correctly. In LM finding, it is higher than the sensitivity but identical with the specificity of the present study. But in case of PM their sensitivity matches with the current study and the specificity is higher than the present study. The 100.0% sensitivity, specificity finding may be due to study of low number (only 12) of cases observed by the above investigators. The results obtained by the present study supported the findings of the investigators and indicated that DQ is highly sensitive as well as specific for detecting MSU crystals.[11]

Dieppe and Swan[5] in a study concluded that the WF LM test has major problems in both its sensitivity and specificity for identification of these crystals. The wet film dried out within a short time and the low concentration and very small size of crystals is partly responsible for the problems in sensitivity. On the contrary, the intracellular crystals were better visualized with DQ stain and long-term preservation of crystals was possible. Moreover, the stained slides showed brilliantly birefringent pathological crystals under PM that was evident even after a year's preservation. So, DQ staining method could be a quick, easy, cost effective procedure with a high sensitivity and specificity as a better diagnostic modality for detection of crystals in synovial fluid.

Conclusions

Wet film polarized microscopy is the 'gold standard' for identification of crystals in synovial fluid and should be used whenever possible. Though PM is not so available, the experience of the study indicates that the stained smear, preferentially Diff Quik method, becomes the preparation of choice for crystal screening in clinical laboratory practice. Since it gives a clear illustration of cells and crystals it may promote and enhance better quality control systems among laboratories. The intracellular crystals are better visualized and long-term preservation is possible. The smears could be preserved and examined later or taken to an outside laboratory where PM facility is available even after a reasonable period of time. This easy, quick and cost-effective procedure is a good alternative where workload is very high for clinicians, pathologist and examination of the WF are often not performed.It offers an extra advantage of cross checking of smear of synovial fluid thus ensure quality control.

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