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Original Research Paper

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UTILITY OF ETHYLENE DIAMINE TETRAACETIC ACID DECALCIFICATION IN BETTERMENT OF QUALITY OF BONE MARROW TREPHINE BIOPSY

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ABSTRACT Bone marrow trephine biopsy(BMTB) is a valuable source of material in diagnosis of neoplastic and non neoplastic hematological conditions.The biopsy after fixation has to be decalcified.Conventionally the tissue is processed without decalcification or decalcified using nitric acid is used or at some centres.Either of this results in many artifects like serrations and folds in the tissue and does not preserve the morphology of the cells rendering in making the diagnosis very difficult.Obtaining BMTB is a painful procedure and cannot be attempted multiple times.By using Ethlyene diamine tetra acetic acid(EDTA) for decalcification the morphology is well preserved and also the proteins making it possible to improve the sensitivity ,specificity and reproducibility of various tests like immunohistochemistry(IHC) and molecular studies.This contributes greatly to the diagnosis ,prognosis ,treatment of patient and provides guidelines to the clinicians in making decisions.The aim of this study was to determine whether EDTA decalcification yielded superior quality slides compared to nondecalcified tissue.

KEYWORDS : EDTA, Bone marrow biopsy, decalcification and artefacts

INTRODUCTION

Specimen of bone marrow when fixed in formalin and processed often results in many artefacts.Serrated sections, folds,loss of marrow tissue while cutting,uneven staining are some of the artefacts.This may result in need for recuts of tissue or even suboptimal reporting.Erroneous reports also may be generated.

EDTA combines with calcium in the outer layer of hydroxyapatite crystals to form soluble nonionic compounds and promote outward transfer of bound calcium.[1] Decalcification of the trephine biopsy using EDTA helps in getting section with least folds ,uniform staining and prevent loss of marrow tissue while cutting the sections.[2]

The density between calcium and paraffin tissue are different and tissue containing calcium cant be sliced directly.If we do directly then it will result in artefacts like loss of marrow tissue while cutting,folds,serrated sections and many other artefacts. This method is characterized by long decalcification time,less damage to bone tissue,better preservation of enzyme activity (alkaline phosphatase) and cell antigenicity.[3]

The aim of this study was to determine whether EDTA decalcification yielded superior quality slides compared to nondecalcified tissue.

MATERIALS AND METHODS

We studied 60 trephine biopsies with prior decalcification and 60 trephine biopsies without decalcification using certain observable parameters and comparables.The procedure we followed was after formalin fixation of the biopsy for 12 hours the tissue is washed in distilled water.Then the tissue is placed in EDTA solution for 24 hours.The solution used was 10 times in order to saturate the tissue.The tissue was then processed routinely embedded in paraffin and sections taken.Adults and children trephine biopsy slides made in surgical pathology laboratory were evaluated.Biopsies having no marrow tissue and the marrow tissue obtained during postmortem were excluded.

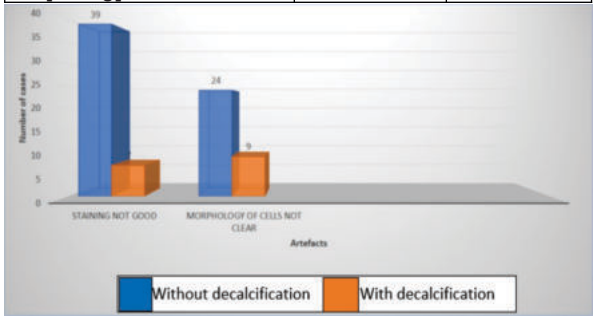
RESULTS

The quality of slides following EDTA decalcification for 24 hrs prior to conventional processing was far superior,thus eliminating the difficulty in reporting and preventing mistakes. Marked improvement was seen with respect to staining, folds, serrated sections, loss of marrow tissue while cutting.

In the biopsies that did not undergo decalcification 29 cases showed poor staining whereas in the biopsies that underwent decalcification only 7 cases showed poor staining quality.Morphology of the cells was not clear in 24 biopsies that did not undergo decalcification and in those biopsies which underwent decalcification only 9 biopsies had poor morphology(Table1 and Fig 1).Folds were seen in 42 of the biopsies which did not undergo decalcification and in only 17 biopsies which underwent decalcification.Serrations were seen in 26 of the biopsies which did not undergo decalcification and in only 9 of the biopsies which underwent decalcification.(Table 2 and Fig 2).Mucinous change was seen in 3 of the biopsies that did not undergo decalcification and in 1 of the biopsy that underwent decalcification.(Table 3 and Fig 3)

Table 1 Showing The Comparison Number Of Biopsies With Respect To Staining And Morphology With Decalcification And Without Decalcification With EDTA

Number of cases (n=120)	With Decalcification (n=60)	Without Decalcification (n=60)
Staining not good	39	7
Morphology of cells not clear	24	9

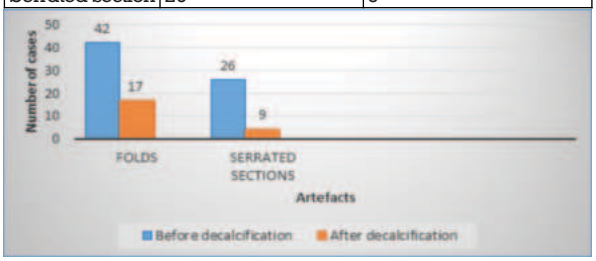


Bar graph 1 Showing The Comparison Number Of Biopsies

With Respect To Staining And Morphology With Decalcification And Without Decalcification With EDTA

Table 2 Showing The Comparison Number Of Biopsies With Respect To Folds And Serrations With Calcification And Without Decalcification With EDTA

	With Decalcification	Without Decalcification
Folds	42	17
Serrated section	26	9



Bar graph 2 Showing The Comparison Number Of Biopsies With Respect To Folds And Serrations With Decalcification And Without Decalcification With EDTA

Table 3 Showing The Comparison Number Of Biopsies Showing Mucinous Change And Crush Artefacts With Decalcification And Without Decalcification With EDTA

	Before decalcification	After decalcification
Mucinous change	3	1
Crush artefact	22	27

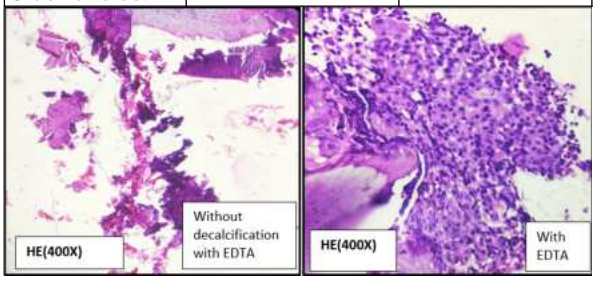


Figure 1 Showing The Comparison Of HE Stained Bone Marrow Biopsy Showing Serrations And Crush Artefacts Without Decalcification(left) And The Serrations And Crush Artifects Having Disappeared After Decalification With EDTA(right)

DISCUSSION

We compared our data with literature. There are studies that mentioned EDTA decalcification is more superior than other decalcifying agents, like our study.

Sung-eun choi et al.(2015)- EDTA was superior in DNA yield and integrity, assessed via DNA extraction. EDTA maintained intact nuclear protein staining on immunohistochemistry.[2]] According to Taqi SA et al.-Incomplete decalcification result in microtome knife and damage to the soft tissue surrounding the calcified area while trimming the paraffin block.[3]However we did not find any study wherein comparison of formalin fixation was done with EDTA fixation, like in our study.

In one of the study by Park and Han in 2016 bone marrow biopsies from 71 patients were decalcified with Hydrochloric acid(HCL) versus EDTA.Differences of decalcification protocols were analysed with respect to Hematoxylin and Eosin staining,Gomoris reticulin staining and immunohistochemistry(IHC) staining and molecular analysis.It was observed that there was remarkably better staining especially in IHC and better preservation of DNA and RNA in biopsies decalcified with EDTA than HCL.[4]

In the era of widespread use of molecular testng for diagnosis and targetted therapy its vital to preserve proteins and nucleic acids for optimal treatment of the patient.The conventional acid based methods used hydrochloric acid and nitric acid allowed rapid turnaround times but would damage the DNA and RNA,so not suitable for molecular diagnosis.[5,6]

In pediatric patients with malignancies like acute lymphoblastic leukemia it is difficult to obtain a bone marrow biopsy.Hence its important to preserve the morphology of the cells as it's a precious material and cannot be obtained again and again.Hence EDTA decalcified biopsy is crucial .[7]

CONCLUSION

A large number of artefacts can be reduced by adequate decalcification of the Trephine enabling correct reporting and preventing delays and mistakes.If institutional turnaround time consideration allows for longer procedure 14% disodium EDTA dihydrate is recommended as tissue decalcified this way is superior and allows high quality protein DNA and RNA.[8,9]Newer studies have used newer techniques to shorten the EDTA decalcification process using magnetic stirrer in a microwave or hot plate at 37-40°C and achieved excellent results.[9]

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