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TAP WATER INDUCES DNA DAMAGE IN THE NUCLEOID OF SMEARED LYMPHOCYTES IN COMET ASSAY - AN INCIDENTAL FINDING

Anatomy

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ABSTRACT

During a routine comet assay procedure that was done in the anatomy lab of a tertiary care institute, as part of a research protocol, an accidental observation was made when tap water was used by chance, instead of distilled water during the electrophoresis step of the procedure(5), that led to increased DNA damage levels in the smeared agarose petrified lymphocytes of children, when compared to the baseline values that were usually expected(2-4). This led to the observation that tap water may be used to detect mild DNA damages in hypoxic children very sensitively. However, using tap water has its own strengths and weaknesses that are explained by the authors in this article

KEYWORDS

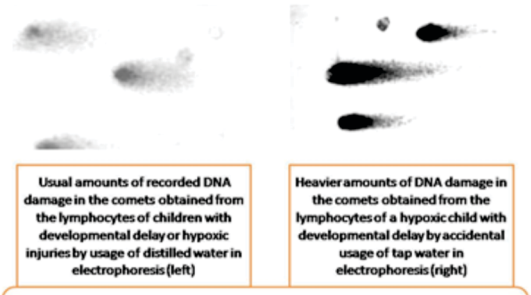
Tap water, DNA, Damage, Comet assay, Nucleoids

INTRODUCTION

The comet assay technique, being one of the oldest pioneering techniques in DNA damage detection is still used in many tertiary care institutions and laboratories as a versatile tool for estimating the amounts of DNA damage in patients who had undergone hypoxic or oxidative stress (1). Being an electrophoresis technique, this comet assay has its own rational step-wise approach in detecting strand breaks in DNA, and alkalinity of water being an essential catalyst for the driving of fragmented DNA supercoils towards the anode. Usually a phosphate or alkaline buffer is added as a catalyst to distilled water to achieve the migration of DNA strands(1,5). But the use of tap water in place of distilled water as an incidental finding for a single case, has caught our attention and revolutionized the findings of our DNA migration values, that is being discussed in this article

FINDING

Increasing the alkalinity in electrophoresis can unwind DNA super-coils at a faster rate, producing excessive DNA damage (1,5). This has been the underlying principle of the comet assay procedure, which is a routinely done test to detect DNA damage levels in the lymphocytes of developmentally delayed children, or children with other hypoxic injuries or children with isolated cleft lip or cleft palate anomalies or even adult patients with an underlying oxidative stress that causes their DNA strands to break (2-4,6). This comet assay being a sensitive technique, is preferred as a screening tool to detect DNA damage before other front-line investigations like karyotyping, PCR or microdot assays are done in the patient (1). During one such comet assay procedure, it was found that while doing routine electrophoresis by comet assay, by chance on one of the case samples, tap water was accidentally used instead of distilled water. At the end of electrophoresis, the slide was dried, fixed and stained. Photos of the comets from that slide was captured and scored. The photographs revealed increased comet tails more than the usual limits (Fig 1). That sample showed a very high level of - DNA migration fold (comet tail length and head diameter of comet), total comet length (100.872 micrometer), % of DNA in comet head (64.95%) and tail (53.71%), when compared to the mean values of other case samples (74.84 mcm,35.58% and 25.54% respectively)(Fig 2).



Usual amounts of recorded DNA damage in the comets obtained from the lymphocytes of children with developmental delay or hypoxic injuries by usage of distilled water in electrophoresis (left)

Heavier amounts of DNA damage in the comets obtained from the lymphocytes of a hypoxic child with developmental delay by accidental usage of tap water in electrophoresis (right)

Fig 1: Comparison between the comet images of the scattered nucleoid fragments of lymphocytes that had undergone electrophoresis using conventional distilled water (left) and by accidental usage of tap water (right)

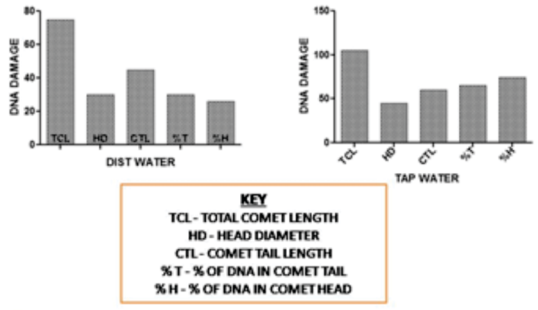


Fig 2: Graph showing the comparison between the comet score parameters of the scattered nucleoid fragments of lymphocytes that had undergone electrophoresis using conventional distilled water (left) and by accidental usage of tap water (right)

The reason behind this which the authors have deciphered is that tap water in some areas, being highly alkaline causes more unwinding of supercoils and removes strand breaks more freely leading to a higher DNA damage. Tap water sometimes contains alkalies of Ca, Mg that disrupt the bases, release supercoils more and cause more strand breaks, thus creating an artificially induced DNA damage (5). Because

of its high alkaline nature, tap water can very sensitively detect minute amounts of DNA damage by detecting the strand breaks. Hence for those cases where only suspected DNA damage occurs, tap water can be used in comet assay. However, it should not be used as a part of the standard protocol in detecting DNA damage because the comet values that are obtained can lead to misinterpretation of results, due to the iatrogenically induced DNA damage and false positive results can be produced if used without applying a correction factor quotient, that can only be obtained if much more studies are done using the tap water for electrophoresis (1,5). This can serve as a "Pandora box" for future anatomists and researchers to do further studies on detection of DNA damage by tap water in comet assay and to standardize its protocol if possible. Hence for the time-being it definitely cannot be accepted as a part of standard protocol unless further studies are done. However, it can just serve as an eye-opener owing to its sharp and sensitive detection capability of DNA strand breaks. Moreover, if tap water is used, it may eliminate the need for using additional buffers like phosphate that are generally used with distilled water in the standard protocol, the reason being tap water in itself is alkaline(1,5).The authors would like to conclude that by eliminating the false errors of high DNA damage and scoring appropriately, the comet assay can be standardized using tap water, especially for a sensitive case detection.

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