Analysis of Normal Range Glomerular Basement Membrane Thickness and Variability with Glomerular Disease

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Abstract

This study has established a normal range of glomerular basement membrane (GBM) thickness for use in clinical diagnosis using existing morphometric GBM measurements. The normal GBM thickness range was determined by examining data from samples fixed in glutaraldehyde or Karnovsky’s fixative which have been processed, embedded and ultrastructurally examined according to the same protocols. There was no significant effect on the estimated mean GBM thickness when tissue was fixed with glutaraldehyde or Karnovsky’s fixative. Clinical cases of thin basement membrane nephropathy and diabetic nephropathy were also analysed to establish thin ranges and thick ranges of GBM measurements, respectively for validated use in ultrastructural reports. There was an adverse effect of reprocessing tissue on estimation of GBM thickness determined using mean GBM thickness data from formalin fixed paraffin wax embedded (FFPE) tissue. Artefactual thinning of the GBM was established in all reprocessed patient samples, which could prevent accurate diagnosis of renal disease involving GBM thickness.

Results

The determined GBM thickness ranges for all patient samples in EM fixative:

- Normal group: 245nm to 337nm
- TBMD group: under 207nm
- Diabetic nephropathy: over 407nm

![Distribution of arithmetic mean GBM measurements in all Control group (normal) patients](image)

No statistical difference in measured GBM thickness fixed with 4% gluteraldehyde and Karnovsky’s Fixative.

Notable difference in measured GBM thickness when reprocessed from FFPE tissue.

Artefactual shrinkage: normal group 24% TBMD group 8%

Diabetic nephropathy group 20%

![Figure 3. The ultrastructural appearance of a normal patient GBM from glutaraldehyde fixed tissue (A) compared to a normal patient GBM from FFPE reprocessed tissue (B). Artefactual thinning is the result of tissue fixation and reprocessing.](image)

Discussion

Ultradeformational morphometry is regularly used as a tool used to provide information on such changes to the glomerular basement membrane. Knowing the ‘normal’ range basement thickness measurements specific to our centre gives the reporting pathologist a baseline from which abnormally thick or thinned GBMs can be identified.

- **Thinned GBM** < 207nm thickness patient likely to have TBMD.
- **Thickened GBM** > 407nm thickness infer early diabetic changes

These ranges and cut off points can be used in ultrastructural commentary as a diagnostic or prognostic aid for renal disease reporting for all fixed renal tissue EM samples.

Differences in reported ranges of estimated GBM thickness are common because specimen handling ultimately affects the ultrastructural morphology.

Variable factors in laboratory protocols:
- tissue fixation
- tissue processing
- reproprocessing and embedding into resin (Edwards, et al., 2009)

Conclusions:
- Fixation with glutaraldehyde or Karnovsky’s fixative has no significant effect on the mean GBM thickness measurements.
- Formalin fixation and reproprocessing protocols produces poor morphological preservation in reproprocessed material.
- Artefactual thinning of the GBM due to shrinkage was noted in all reproprocessed FFPE tissue.
- Determined ranges and cut off points for fixed samples can not be applied to reproprocessed FFPE tissue.

The ultrastructural commentary must consider the handling of the specimen prior to morphometry of the GBM and reproprocessed tissue must not be used to diagnose abnormally thinned or thickened GBMs alone.

Further work could include a wider study of reproprocessed tissue and fixed processed tissue from the same patients to directly compare processing effects and determine GBM thickness ranges for FFPE tissue.

Key References


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Introduction

Morphometric measurement of the GBM thickness by electron microscopy is necessary for the diagnosis of both thin basement membrane disease (TBMD) and diabetic nephropathy (Darouch, S. et al., 2010). There is no well-defined standard criteria which can be applied locally to describe the lower and upper limits of ‘normal’ range to determine if the GBM is thin or thick.

Different diagnostic centres report a variable range of normal thickness measurements, most likely due to differences in tissue fixation and processing (Naar, S. et al., 2007).

Information gathered from this study will demonstrate if fixation, processing, and reproprocessing protocols influence the mean GBM thickness estimated by ultrastructural morphometry.

Methods

- 302 patient renal samples obtained from three centres: University Hospitals of Leicester Royal Free Hospital in London St. James’ University Hospital in Leeds
- Normal/Control group (n=183), TBMD group (n=36) and Diabetic Nephropathy (n=183).
- EM samples fixed in 4% Glutaraldehyde or Karnovsky’s Fixative, washed in Sorensen’s phosphate buffer, post fixed in 2% buffered Osmium Tetroxide, processed through increasing grade ethanol, into acetone, embedded in araldite resin and left overnight in a 70°C oven to allow polymerisation. FFPE samples were reprocessed by de-waxing with xylene and rehydration in decreasing graded ethanol prior to processing through to resin.
- Ultrastructural examination of stained electron microscopy grids previously performed using a JEOL 1400 or JEOL 1400plus electron microscope. Calibrated digital images taken using a digital camera using AMT TEM camera software.
- Mean GBM thickness measurements determined from archive transmission electron microscopy images by averaging morphometric perpendicular measurements taken from the endothelial cell basement membrane to epithelial podocyte basement.

![Figure 1. Frequency histogram showing distribution of normal patient’s GBM thickness measurements (nm) of samples fixed in glutaraldehyde and Karnovsky’s fixative.](image)

![Figure 2. TEM images of glomerular basement membranes of patient samples fixed in glutaraldehyde and Karnovsky’s fixative. A - the GBM of a patient with normal GBM and B - shows the thinned GBM of a patient diagnosed with diabetic nephropathy.](image)