

## QUANTITATIVE ESTIMATION OF RUFFLING OF LUMINAL SURFACE MEMBRANE OF ENDOTHELIAL CELLS IN THE COLONIC CAPILLARIES OF MONKEYS INFECTED WITH SHIGA TOXIN PRODUCING *ESCHERICHIA COLI* O157:H7

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Shiga toxin producing *Escherichia coli* O157:H7 (STEC) is known to cause morphological changes in the microvasculature of the colon of monkeys. The objective of the study was to estimate the difference in the ruffling of the luminal surface membrane of endothelial cells of capillaries from monkeys infected with STEC and control animals.

A total of 24 capillary profiles from the colon of two non-diarrhoeic monkeys given non-pathogenic *E. coli* were selected. One monkey was sacrificed at 6 hours and other at 24 hours after administering the pathogens. Sixty five capillaries from the colon of diarrhoeic monkeys given STEC were selected. Of these, 37 capillaries were from monkeys sacrificed at six hours post infection (PI) and 28 were from monkeys sacrificed at 24 hours PI. Onset of diarrhoea in the infected group was observed after 24 hours. The perimeter of the lumen (P) and area of the lumen (A1) for each capillary were determined using a computer programme. The Compactness Index (CI) of the lumen,  $P/\sqrt{A1}$  was calculated for each capillary and used as an indicator of ruffling of luminal membrane. Higher values of the CI indicate more ruffling. Group differences in the CI were estimated using an independent sample T-test and one-way analysis of variance (ANOVA). P values less than 0.05 were considered significant.

The mean CI in controls ( $8 \pm 3$ ) and STEC treated monkeys ( $11.6 \pm 5.7$ ) were significantly different ( $P = 0.001$ ) from each other. The CI in monkeys sacrificed at 6 and 24 hours was  $11.4 \pm 5.4$  and  $11.7 \pm 6.1$ , respectively. The one-way ANOVA showed significant differences between the three groups ( $P = 0.016$ ) with Bonferroni post-hoc tests showing significant differences in the CI between control capillaries and capillaries from STEC infected monkeys at both time points. However, there was no significant difference in the CI between the capillaries of STEC-infected monkeys at 6 hours and 24 hours PI.

The significantly higher CI in capillaries from STEC-infected monkeys compared to controls suggests that STEC contributes to ruffling of the luminal membrane of the endothelial cells. This change is well developed at 6 hours PI and is maintained till 24 hours PI. This study reports a novel way of quantifying the ruffling of the luminal membrane of endothelial cells in injury or perturbation.