

Appendix IV: Scoring of P16 gene expression immuno-staining.

Positive results were reported with regard to:

Site of staining: as either nucleus or cytoplasm

Intensity of staining: graded from 0(negative) to grade 3(strong)

Percentage of tumor cells staining: at (x40) was graded from grade 0 (negative) to grade IV.

Immuno-reactivity of P16 protein expression was graded into four groups according to the fraction of positive tumor cells and intensity of positive of positive staining.

Grade III: strong/high immuno-reactivity in more than 25% of tumor cells.

Grade II: moderate immuno-reactivity in more than 25% of tumor cells

Or strong immuno-reactivity in less than 25% of the tumor cells.

Grade I: Low/ weak immuno-reactivity in more than 25 % of the tumor cells

Or Moderate immuno-reactivity in less than 25% of the tumor cells.

Grade 0: lack of immuno-staining (negative)

Or weak immuno-staining in less than 25% of the tumor cells

Grade II and III were considered positive whereas grades 0 and I as normal tissue.