**Department of Medical Epidemiology and Biostatistics**

# Risk Factors for Esophageal Squamous Cell Carcinoma

AKADEMISK AVHANDLING
som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i Lennart Nilsson, Nobels väg 15A

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**ABSTRACT**

**The etiology of esophageal squamous cell carcinoma (ESCC) in the high risk areas is largely unknown and a few environmental risk factors which have been identified do not explain its oddly high incidence in esophageal cancer belt. The aims of this thesis were to investigate the association of opium/tobacco consumption, serologic gastric atrophy, gastric mucosa-associated microbiota and contact with farm animals with the risk of ESCC.**

**Methods: The population of Golestan Province in northeastern Iran has very high rates of ESCC. From 2003 to 2007, we administered a validated structured questionnaire to 300 incident ESCC cases and 571 controls. Controls were matched to cases for neighborhood of residence, age (± 2 years), and sex. We measured serum pepsinogen I and II among 293 incident cases and 524 matched controls. Conditional logistic regression models were applied to calculate odds ratios (ORs) and 95% confidence intervals (CIs) adjusted for potential confounders. Furthermore we obtained a frozen gastric tissue biopsy from subjects with ESCC, esophageal squamous dysplasia, mid esophagus esophagitis, and age/sex-matched endoscopy clinic controls with healthy esophagus. To characterize bacterial lineage present in gastric mucosa, we performed a multiplex sequencing with GS-FLX Titanium targeting 16S rRNA.**

**Risk of ESCC was increased in those who used opium only (OR= 2.12, 95% CI: 1.21 – 3.74), and in those who used both tobacco and opium (2.35, 95% CI: 1.50 – 3.67). All forms of tobacco use (cigarettes, hookah, and nass) were associated with higher ESCC risk.**

**Gastric atrophy (defined by a validated criterion, pepsinogen I <55 µg/dl) was associated with a two-fold increased risk (OR = 2.01, 95% CI: 1.18- 3.45) of ESCC in the absence of nonatrophic pangastritis (defined as pepsinogen II<11.8 µg/dl).**

**Sequencing of 16s rRNA in gastric biopsy samples resulted 2075 operational taxonomic units (OTUs). Conditional logistic regression model based on principal coordinate analysis (PCoA) showed a marginal variation in pattern of gastric microbiota using Unifrac (p=0.004) and weighted Unifrac distances (p= 0.018) between subjects with esophageal cancer or dysplasia and controls. No such difference between subjects with mid-esophagitis and controls was observed.**

**Among four groups of farm animals (equines, ruminants, domestic canine and poultry) contact with ruminants was associated with an 8-fold increase in risk of ESCC. This association stayed stable when duration and level of contact were considered.**

**Though opium and tobacco consumption are associated with the risk of ESCC in the study area, they do not explain the extreme high incidence in northern Iran. Changes in gastric environment may link to ESCC risk based on observations that fundal atrophy may increase the risk for ESCC and pattern of gastric microbiota differs in patients with esophageal squamous dysplasia (and ESCC) from subjects with normal esophagus. The observed relationship between lifelong contact with ruminants and ESCC needs further investigation.**

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