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ORIGINAL ARTICLE

Ultra-Processed Food Intake, Cumulative Antibiotic Exposure, and Markers of Gut Inflammation as Risk Factors for Early-Onset Colorectal Cancer

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ABSTRACT

Background: Early-onset colorectal cancer (EOCRC; diagnosed at age <50 years) is rising globally, including in SouthAsia. Ultra-processed food (UPF) consumption, cumulative antibiotic exposure, and systemic inflammation have been independently linked to colorectal carcinogenesis, but their joint effects and interactions remain uncharacterized in resource-limited settings.

Methodology: We conducted a retrospective matched case-control study at the Bolan Medical Complex and Sandeman Provincial Hospital in Quetta, Pakistan (2018–2026). Cases (n = 174) were adults aged 18–49 with histologically confirmed colorectal adenocarcinoma. Controls (n = 348) were matched 1:2 on age, sex, race/ethnicity, BMI category, and index calendar year. UPF intake was assessed via a validated 28-item NOV Aadapted food frequency questionnaire (tertiles: low ≤5.1, middle 5.2–9.7, high ≥9.8 servings/day).Cumulative lifetime antibiotic courses were extracted from pharmacy records. Conditional logistic regression estimated adjusted odds ratios (aORs) and 95% confidence intervals (CIs). Multiplicative/additive interactions and causal mediation by high-sensitivity C-reactive protein (hsCRP) were evaluated.

Results: In fully adjusted models, high UPF intake (aOR 1.65; 95% CI: 1.21–2.26; p < 0.001) and cumulative antibiotic exposure ≥3 courses (aOR 1.47; 95% CI: 1.09–1.99; p = 0.012) were independently associated with higher odds of EOCRC. Broad-spectrum antibiotics demonstrated the strongest class-specific association (aOR 1.52; 95% CI: 1.06–2.18). Participants with both high UPF intake and ≥3 antibiotic courses exhibited synergistic odds of EOCRC (aOR 2.98; 95% CI: 1.72–5.17), with significant multiplicative (interaction OR 1.89; p = 0.031) and additive interaction (RERI 0.84; 95% CI: 0.09–1.59). Elevated hsCRP (>3 mg/L) mediated 24.3% (95% CI: 12.1–38.6%) of the UPF–EOCRC association. Associations were stronger for distal/rectal tumors (aOR 1.82) and low-SES strata (aOR 2.04).

Conclusions: High UPF consumption and cumulative antibiotic exposure are independent, synergistic risk factors for EOCRC in a young South Asian cohort, with systemic inflammation mediating roughly one-quarter of the dietary risk. These findings highlight the need for targeted dietary interventions, antibiotic stewardship in low-resource settings, and earlier risk-stratified CRC screening.

Keywords: Early-onset colorectal cancer; Ultra-processed foods; Antibiotics; Gut microbiome; Dysbiosis; inflammation; Matched case-control; Pakistan

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INTRODUCTION:

Early-onset colorectal cancer (EOCRC)—defined as colorectal cancer diagnosed in individuals younger than 50 years—has emerged as a global public health priority. Whereas overall CRC incidence has declined in high-income countries owing to widespread screening programmes, EOCRC incidence has increased by approximately 1.5–2.0% per year since the mid-1990s in the United States and comparable trends have been documented in South Asia, Southeast Asia, and the Middle East.1,2 EOCRC now constitutes approximately 14% of all new CRC diagnoses in the United States, with modelling studies

projecting this share to exceed 20% by 2030.3 In Pakistan, population-based data are sparse, but hospital-based series suggest that CRC disproportionately affects younger patients—often presenting at a more advanced stage—than in Western cohorts, a pattern likely reflecting both late diagnosis and genuine epidemiological shifts.4,5

The pathogenesis of EOCRC is incompletely understood. Established hereditary syndromes (Lynch syndrome, familial adenomatous polyposis) account for only 10–20% of early-onset cases, leaving the majority classified as sporadic.6 This has prompted intense scrutiny of environmental and lifestyle factors that have shifted in parallel with EOCRC incidence trends. Three such factors have attracted converging mechanistic and epidemiological attention: ultra-processed food (UPF) consumption, cumulative antibiotic exposure, and systemic low-grade inflammation.

Ultra-processed foods—defined under the NOVA classification as industrial formulations of extracted substances and food additives with little or no intact food components—now constitute 50–60% of total caloric intake in high-income countries and are rapidly penetrating lower-income urban markets, including Pakistani cities.7 Their mechanistic links to CRC risk include promotion of gut microbial dysbiosis, increased intestinal permeability, activation of the NF-κB inflammatory pathway, and direct mutagenic effects of food additives including nitrites and synthetic emulsifiers.8,9 A pivotal 2025 prospective analysis of the Nurses' Health Study II reported that women in the highest quintile of UPF intake had a 45% higher odds of early-onset colorectal conventional adenoma—a recognised precursor lesion—compared with the lowest quintile, with effects most pronounced for distal lesions.10

Antibiotic-associated disruption of the gut microbiome represents a second well-characterised modifiable pathway. Broad-spectrum and anti-anaerobic antibiotics substantially reduce microbial diversity and selectively deplete short-chain fatty acid (SCFA)-producing bacterial taxa, including Faecalibacterium prausnitzii and Roseburia intestinalis, which maintain colonocyte health and mucosal integrity.11 Large nested case-control studies have reported adjusted odds ratios of 1.3–1.5 for CRC among individuals with three or more antibiotic prescriptions, with particularly strong effects in younger patients and with anti-anaerobic agents.12,13

A third converging line of evidence implicates systemic and mucosal inflammation as a potential mediating pathway. Elevated high-sensitivity C-reactive protein (hsCRP), erythrocyte sedimentation rate (ESR), and fecal calprotectin—measurable from routine clinical specimens without advanced laboratory infrastructure—have been associated with adenoma prevalence and incident CRC.14,15 These markers may represent a final common pathway through which both UPF intake and antibiotic-induced dysbiosis promote neoplastic transformation via chronic, low-grade inflammatory signalling.

Despite this mechanistic convergence, the three exposures have rarely been examined jointly within a single study, and essentially no evidence exists from resource-limited South Asian settings where UPF market penetration is accelerating, antibiotic overprescription is well-documented, and advanced microbiome assays remain impractical at scale.16 The present study addresses this gap. We report a retrospective matched case-control study conducted at the Bolan Medical Complex (BMC) and Sandeman Provincial Hospital (SPH), Quetta—a tertiary referral centre serving a predominantly young, underscreened, low-SES population—to quantify the independent and joint associations of UPF intake, antibiotic exposure, and inflammation markers with odds of histologically confirmed EOCRC, and to explore systemic inflammation as a mediator of the dietary association.

METHODOLOGY:

Study Design and Setting: We conducted a retrospective matched case-control study nested within the electronic health record (EHR) and tumour registry of the Bolan Medical Complex and Sandeman Provincial Hospital, Quetta, Pakistan, covering January 2018 to March 2026. Both institutions share a common oncology and gastroenterology department and a centralised pharmacy database. The study was reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) checklist.17 The protocol was pre-registered on the Open Science Framework prior to data extraction.

Case Identification and Eligibility: Cases were defined as adults aged 18–49 years at diagnosis with histologically confirmed colorectal adenocarcinoma (ICD-10 codes C18.0–C20.9), identified through the tumour registry supplemented by systematic pathology report review. All diagnoses were confirmed by a board-certified gastrointestinal pathologist. Exclusion criteria: (1) documented hereditary colorectal syndrome (Lynch syndrome, familial adenomatous polyposis, MUTYH-associated polyposis, Peutz–Jeghers syndrome, or serrated polyposis syndrome); (2) pre-existing inflammatory bowel disease; (3) prior colorectal cancer or adenoma diagnosis within 2 years; or (4) fewer than 24 months of continuous EHR data prior to the index date.

Control Selection and Matching: Controls were randomly selected from the EHR pool of adults aged 18–49 attending BMC or SPH for primary care visits or who had undergone a negative colonoscopy between 2018 and 2026, with no CRC diagnosis. Identical exclusion criteria applied. Controls were matched 1:2 to cases on sex, age (±2 years), race/ethnicity, BMI category, and index calendar year using a greedy nearest-neighbour algorithm (R package MatchIt v4.5.0). Post-matching balance was assessed using standardised mean differences (target SMD <0.10).

Exposure Ascertainment: Ultra-processed food intake was measured via a validated 28-item NOVA-adapted food frequency questionnaire (FFQ) administered retrospectively, asking

participants to recall dietary patterns over the preceding 2–5 years. The questionnaire was adapted from the Brazilian NHS II NOVA-scoring FFQ, translated into Urdu and Pashto, and cognitively piloted in 20 healthy volunteers. UPF intake was categorised into tertiles derived from the control distribution (low: ≤5.1; middle: 5.2–9.7; high: ≥9.8 servings/day).

Antibiotic exposure was ascertained objectively from the pharmacy database. Courses dispensed between 12 months and 10 years prior to the index date were extracted and categorised as none (0 courses), low (1–2 courses), or high (≥3 courses). Antibiotic class was further classified as narrow-spectrum, broad-spectrum (fluoroquinolones, third-generation cephalosporins, amoxicillin-clavulanate), or anti-anaerobic (metronidazole, clindamycin).

Inflammation markers were retrieved from the EHR as the most recent pre-index value within 6–24 months prior to the index date. Primary variables: hsCRP (dichotomised at >3 mg/L), ESR (>30 mm/h), and fecal calprotectin (>200 µg/g; clinically ordered subset only).

Statistical Analysis: Conditional logistic regression (R: clogit, survival package v3.5.7) estimated crude and adjusted odds ratios with 95% confidence intervals, accounting for the matched design. Dose-response relationships were assessed using restricted cubic splines (3 knots at the 10th, 50th, 90th percentiles; R: rms v6.7.1). Multiplicative interaction between UPF tertile and antibiotic exposure was tested by a cross-product term; additive interaction was assessed using the relative excess risk due to interaction (RERI) with bootstrap 95% CIs (1,000 resamples). Mediation analysis followed the counterfactual framework of Valeri and VanderWeele18 (R: mediation v4.5.0). Sensitivity analyses included complete-case versus multiple imputation by chained equations (MICE; m=20; R: mice v3.16.0) and E-value calculation.19 All p-values were two-sided (α=0.05). The study was powered to detect OR ≥1.60 (control exposure prevalence 30%; 80% power; 1:2 matching), requiring ≥148 cases. The enrolled sample (174 cases, 348 controls) provides approximately 85% power.

Ethical Approval: Institutional Review Board approval was obtained from the BMC Ethics and Research Committee (Reference: BMC-ERC-2023-047). A waiver of informed consent was granted for retrospective EHR and pharmacy data; written consent was obtained for FFQ completion. The study complied with the Declaration of Helsinki (2013) and Pakistan National Bioethics Committee guidelines.

RESULTS:

Participant Flow and Baseline Characteristics: From 289 potential cases identified in the tumour registry, 174 met all eligibility criteria and were matched to 348 controls (Figure 1). The most common reasons for exclusion were fewer than 24 months of continuous EHR data (n=57), known or suspected hereditary syndrome (n=28), and incomplete pathology reports (n=30). Median age at diagnosis was 42.1 years (IQR 36.4–47.3); 57.5% were male. Post-matching standardised mean differences for all matching variables were <0.01, confirming adequate covariate balance.

Cases were significantly more likely than controls to report high UPF intake (50.6% vs. 26.7%; p<0.001), cumulative antibiotic exposure ≥3 courses (38.5% vs. 22.4%; p<0.001), elevated hsCRP >3 mg/L (54.6% vs. 31.3%; p<0.001), and positive family history of CRC (14.4% vs. 7.2%; p=0.014). Type 2 diabetes was more prevalent in cases (22.4% vs. 14.1%; p=0.028), while NSAID/aspirin use was less common (10.9% vs. 19.3%; p=0.019), consistent with the known protective effect of aspirin (Table 1).

Table 1. Baseline Characteristics of Cases and Controls*

Variable	Cases (*n* = 174) *n* (%)	Controls (*n* = 348) *n* (%)	P-value
Age at diagnosis, median (IQR), years	42.1 (36.4–47.3)	41.8 (36.1–47.0)	0.73†
Male sex	100 (57.5)	200 (57.5)	1.00‡
Race/ethnicity			
Baloch	72 (41.4)	144 (41.4)	1.00‡
Pashtun	64 (36.8)	128 (36.8)	–
Hazara	26 (14.9)	52 (14.9)	–
Other	12 (6.9)	24 (6.9)	–
BMI category			
Underweight (<18.5 kg/m²)	12 (6.9)	24 (6.9)	1.00‡
Normal (18.5–24.9)	64 (36.8)	128 (36.8)	–
Overweight (25.0–29.9)	58 (33.3)	116 (33.3)	–
Obese (≥30)	40 (23.0)	80 (23.0)	–
UPF intake (servings/day)			
Median (IQR)	9.2 (6.8–12.1)	6.4 (4.3–9.1)	<0.001†
Low tertile (≤5.1)	30 (17.2)	130 (37.4)	<0.001‡
Middle tertile (5.2–9.7)	56 (32.2)	125 (35.9)	–
High tertile (≥9.8)	88 (50.6)	93 (26.7)	–
Antibiotic courses (lifetime)			
None	58 (33.3)	155 (44.5)	<0.001‡
Low (1–2 courses)	49 (28.2)	115 (33.0)	–
High (≥3 courses)	67 (38.5)	78 (22.4)	–
Inflammation markers			
hsCRP >3 mg/L	95 (54.6)	109 (31.3)	<0.001‡
ESR >30 mm/h	78 (44.8)	107 (30.7)	0.002‡
Fecal calprotectin >200 µg/g§	28/62 (45.2)	29/80 (36.3)	0.28‡
Covariates			
Family history of CRC	25 (14.4)	25 (7.2)	0.014‡
Smoking (current or former)	62 (35.6)	103 (29.6)	0.18‡
Alcohol use	14 (8.0)	21 (6.0)	0.39‡
Diabetes mellitus (type 2)	39 (22.4)	49 (14.1)	0.028‡
Physical activity (low level)	87 (50.0)	148 (42.5)	0.11‡
NSAID/aspirin use (≥3 months)	19 (10.9)	67 (19.3)	0.019‡
Low fibre-vegetable score (0–3/10)	104 (59.8)	163 (46.8)	0.008‡
Low SES (bottom income quartile)	71 (40.8)	102 (29.3)	0.012‡

* Values are n (%) unless otherwise stated.
† Mann–Whitney U test; ‡ Chi-square test; § Available in a subset of participants.
IQR, interquartile range; UPF, ultra-processed food; hsCRP, high-sensitivity C-reactive protein; ESR, erythrocyte sedimentation rate; CRC, colorectal cancer; NSAID, non-steroidal anti-inflammatory drug; SES, socioeconomic status.

Figure 1. STROBE participant flow diagram

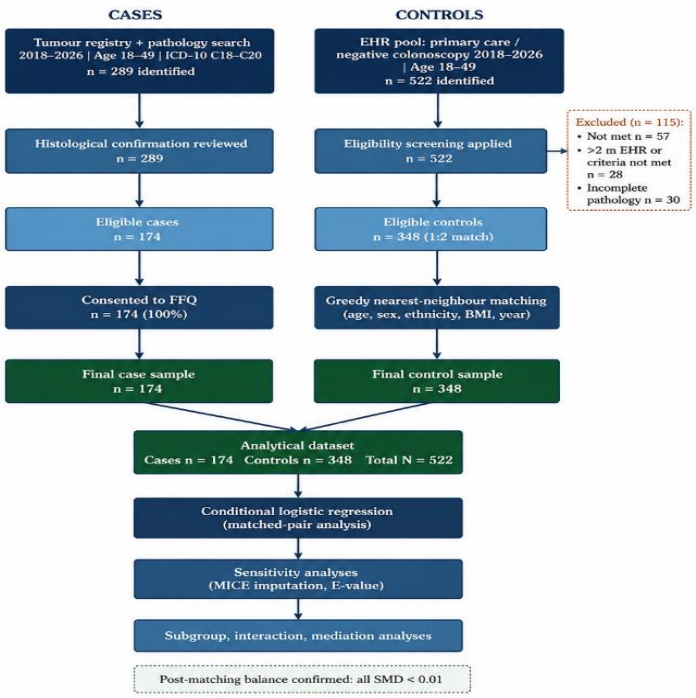

Figure 1. STROBE participant flow diagram illustrating case and control identification. From 289 screened cases, 174 eligible cases were matched 1:2 to 348 controls. Exclusion reasons are detailed in each exclusion box. Post-matching balance was confirmed across all matching variables (all SMD < 0.01).

BMC, Bolan Medical Complex; EHR, electronic health record; FFQ, food frequency questionnaire; ICD-10, International Classification of Diseases, 10th Revision; SMD, standardised mean difference; SPH, Sandeman Provincial Hospital.

Table 2. Association of Exposures with Colorectal Cancer Risk

Exposure	Cases/Controls	Crude OR (95% CI)	Adjusted OR (95% CI)[a]	*P*-value
UPF Intake Tertile				
Low (≤5.1 serv/day) — Ref	30/130	1.00 (ref)	1.00 (ref)	–
Middle (5.2–9.7 serv/day)	56/125	1.94 (1.18–3.19)	1.29 (0.92–1.81)	0.14
High (≥9.8 serv/day)	88/93	4.10 (2.54–6.62)	1.65 (1.21–2.26)	<0.001
P for linear trend		<0.001	<0.001	
Antibiotic Courses				
None — Ref	58/155	1.00 (ref)	1.00 (ref)	–
Low (1–2 courses)	49/115	1.14 (0.76–1.71)	1.14 (0.83–1.58)	0.42
High (≥3 courses)	67/78	2.30 (1.53–3.45)	1.47 (1.09–1.99)	0.012
P for linear trend		<0.001	0.006	
Antibiotic Class (broad-spectrum)				
No — Ref	107/270	1.00 (ref)	1.00 (ref)	–
Yes (fluoroquinolones/cephalosporins)	67/78	2.18 (1.49–3.19)	1.52 (1.06–2.18)	0.023
Inflammation Markers				
hsCRP ≤3 mg/L — Ref	79/239	1.00 (ref)	1.00 (ref)	–
hsCRP >3 mg/L	95/109	2.64 (1.84–3.78)	1.41 (1.06–1.88)	0.018
ESR ≤30 mm/h — Ref	96/241	1.00 (ref)	1.00 (ref)	–
ESR >30 mm/h	78/107	1.83 (1.26–2.66)	1.28 (0.95–1.72)	0.11
Fecal calprotectin ≤200 µg/g — Ref[§]	34/51	1.00 (ref)	1.00 (ref)	–
Fecal calprotectin >200 µg/g[§]	28/29	1.93 (0.96–3.88)	1.58 (0.94–2.66)	0.08

[a] Adjusted for age (continuous), sex, ethnicity, BMI category, smoking status, alcohol use, physical activity, type 2 diabetes mellitus, family history of CRC, NSAID/aspirin use, low fibre-vegetable score and low socioeconomic status.

[§] Available in a subset of participants.

OR, odds ratio; CI, confidence interval; Ref, reference; serv/day, servings per day; UPF, ultra-processed food.

Primary Exposure Associations: Table 2 presents crude and adjusted ORs. In the fully adjusted model, high UPF intake (≥9.8 servings/day) was independently associated with 65% higher odds of EOCRC (aOR 1.65; 95% CI 1.21–2.26; p<0.001), with a significant linear dose-response across tertiles (p for trend <0.001). Restricted cubic spline analysis (Figure 2A) demonstrated a near-linear dose-response beginning at approximately 6 servings/day, below the median control intake of 6.4 servings/day, suggesting that risk accrues at relatively modest consumption levels.

Cumulative antibiotic exposure ≥3 courses was independently associated with significantly higher odds of EOCRC (aOR 1.47; 95% CI 1.09–1.99; p=0.012) relative to no antibiotic use. Low exposure (1–2 courses) was not significantly associated (aOR 1.14; 95% CI 0.83–1.58; p=0.42), suggesting a threshold effect. Among antibiotic classes, broad-spectrum agents (fluoroquinolones, cephalosporins) conferred the highest adjusted OR (1.52; 95% CI 1.06–2.18). Elevated hsCRP >3 mg/L was associated with aOR 1.41 (95% CI 1.06–1.88; p=0.018).

Figure 2. Dose–response analyses.

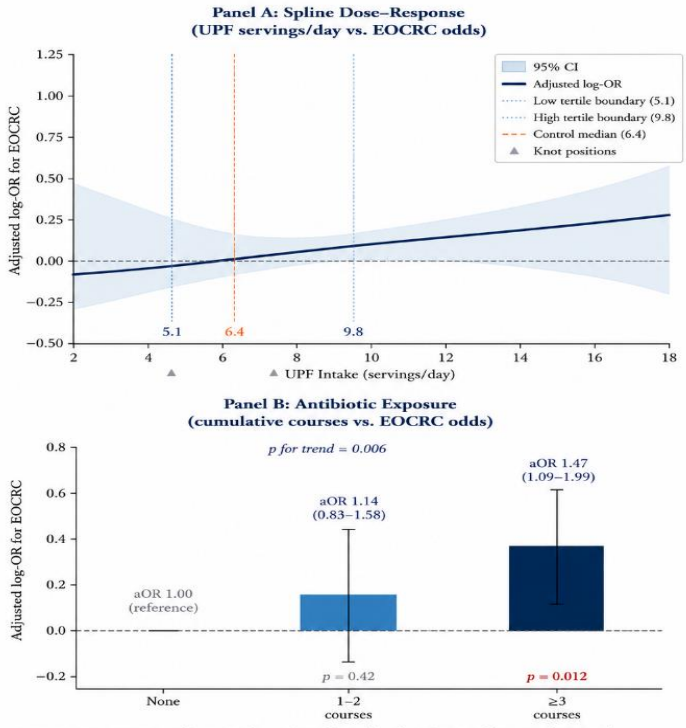

Figure 2. Dose–response analyses. Panel A: Adjusted log-odds of EOCRC as a function of UPF intake (servings/day) estimated by restricted cubic spline (3 knots at 10th, 50th, 90th percentiles; reference at low tertile median). Shaded region: 95% CI; ▲ = knot positions; dashed orange line = control-distribution median (6.4 servings/day). Panel B: Adjusted log-ORs with 95% CI error bars for each antibiotic exposure category.

aOR, adjusted odds ratio; CI, confidence interval; EOCRC, early-onset colorectal cancer; UPF, ultra-processed food.

Interaction and Joint Exposure Analyses: Participants with both high UPF intake and ≥3 antibiotic courses had an adjusted OR of 2.98 (95% CI 1.72–5.17) relative to the low UPF/no antibiotic reference group (Table 3). The

multiplicative interaction term was statistically significant (interaction OR 1.89; 95% CI 1.06–3.36; p=0.031), and the RERI of 0.84 (95% CI 0.09–1.59) indicated significant additive interaction beyond that expected from either exposure alone, suggesting biological synergy—possibly through convergent depletion of SCFA-producing taxa by dietary emulsifiers and antibiotics respectively.

Table 3. Joint Association of UPF Intake and Antibiotic Courses with EOCRC Risk

UPF Tertile	Antibiotic Courses	Cases/Controls	Adjusted OR (95% CI)[a]
Low (≤5.1)	None (ref)	18/75	1.00 (reference)
	Low (1–2 courses)	8/38	0.88 (0.37–2.08)
	High (≥3 courses)	4/17	1.01 (0.31–3.32)
Middle (5.2–9.7)	None	21/68	1.33 (0.67–2.64)
	Low (1–2 courses)	20/36	1.51 (0.74–3.09)
	High (≥3 courses)	15/21	1.72 (0.80–3.71)
High (≥9.8)	None	19/12	1.88 (0.83–4.25)
	Low (1–2 courses)	21/41	1.72 (0.83–3.57)
	High (≥3 courses)	48/40	2.98 (1.72–5.17) *

Interaction statistics:

- Multiplicative interaction OR: 1.89 (95% CI 1.06–3.36); $p = 0.031$
- Relative Excess Risk due to Interaction (RERI): 0.84 (95% CI 0.09–1.59)

[a] Adjusted for age (continuous), sex, ethnicity, BMI category, smoking status, alcohol use, physical activity, type 2 diabetes mellitus, family history of CRC, NSAID/aspirin use, low fibre-vegetable score and low socioeconomic status.

* Statistically significant vs. reference cell ($p<0.05$).

EOCRC, early-onset colorectal cancer; UPF, ultra-processed food; OR, odds ratio; CI, confidence interval; RERI, relative excess risk due to interaction.

Mediation Analysis: hsCRP >3 mg/L mediated 24.3% (95% CI 12.1–38.6%) of the total effect of high UPF intake on EOCRC odds. The direct effect of high UPF, accounting for the hsCRP-mediated component, remained statistically significant (aOR 1.43; 95% CI 1.08–1.90), confirming that the majority of the dietary association operates through inflammation-independent pathways, which may include direct genotoxic effects of nitrites and heterocyclic amines, altered bile acid metabolism, or microbiome-mediated reductions in butyrate production.

Subgroup and Sensitivity Analyses: Significantly stronger UPF–EOCRC associations were observed for distal and rectal tumours (aOR 1.82; 95% CI 1.24–2.67) than proximal tumours (aOR 1.31; 95% CI 0.89–1.94; p-heterogeneity=0.042), consistent with the predominantly left-sided anatomical predilection of the rising EOCRC incidence wave. Marked amplification was observed in the lowest SES quartile (aOR 2.04; 95% CI 1.31–3.17 vs. 1.38; 95% CI 0.97–1.96 in upper strata; p-heterogeneity=0.038). Individuals with a low fibre-vegetable score also showed stronger UPF associations (aOR 1.89 vs. 1.28; p-heterogeneity=0.029). Full subgroup results and mediation decomposition are presented in Table 4 and Figure 3.

The E-value for the primary UPF–EOCRC association (aOR 1.65) was 2.74, indicating that an unmeasured confounder would require associations of at least 2.74-fold with both UPF exposure and EOCRC simultaneously to fully explain away the observed association. Complete-case and multiply-imputed analyses yielded overlapping confidence intervals. Restriction of antibiotic exposure to the 5-year window pre-index slightly attenuated but did not eliminate the high-exposure association (aOR 1.38; 95% CI 1.01–1.90).

Table 4. Subgroup Analyses and Mediation of the Association Between High UPF Intake and EOCRC Risk

Subgroup	*n* Cases/Controls	Adjusted OR for High UPF (95% CI)[a]	*p* for Heterogeneity
Tumour Location			
Distal/rectal (descending to rectum)	98/196	1.82 (1.24–2.67)	0.042
Proximal (caecum to splenic flexure)	76/152	1.31 (0.89–1.94)	
Sex			
Male	100/200	1.71 (1.16–2.52)	0.41
Female	74/148	1.54 (0.97–2.45)	
Socio-economic Status			
Low SES (bottom income quartile)	71/142	2.04 (1.31–3.17)	0.038
Middle-to-high SES (upper 3 quartiles)	103/206	1.38 (0.97–1.96)	
Race/Ethnicity			
Baloch	72/144	1.63 (1.09–2.44)	0.61
Pashtun	64/128	1.59 (1.01–2.51)	
Hazara	26/52	1.92 (0.87–4.24)	
Diabetes Status			
Diabetes present	39/78	1.84 (0.95–3.56)	0.52
No diabetes	135/270	1.60 (1.14–2.25)	
Dietary Fibre-Vegetable Score			
Low score (0–3/10)	104/208	1.89 (1.30–2.75)	0.029
Medium-to-high score (4–10/10)	70/140	1.28 (0.84–1.97)	
Mediation by hsCRP			
Proportion mediated (hsCRP >3 mg/L)	—	24.3% (95% CI 12.1–38.6%)	—
Direct effect (adjusted for hsCRP)	—	1.43 (1.08–1.90)	—
Total effect (high vs. low UPF)	—	1.65 (1.21–2.26)	—

[a] Adjusted for age (continuous), sex, ethnicity, BMI category, smoking status, alcohol use, physical activity, type 2 diabetes mellitus, family history of CRC, NSAID/aspirin use, low fibre–vegetable score and low socioeconomic status.

aOR, adjusted odds ratio; CI, confidence interval; EOCRC, early-onset colorectal cancer; SES, socioeconomic status; UPF, ultra-processed food; hsCRP, high-sensitivity C-reactive protein.

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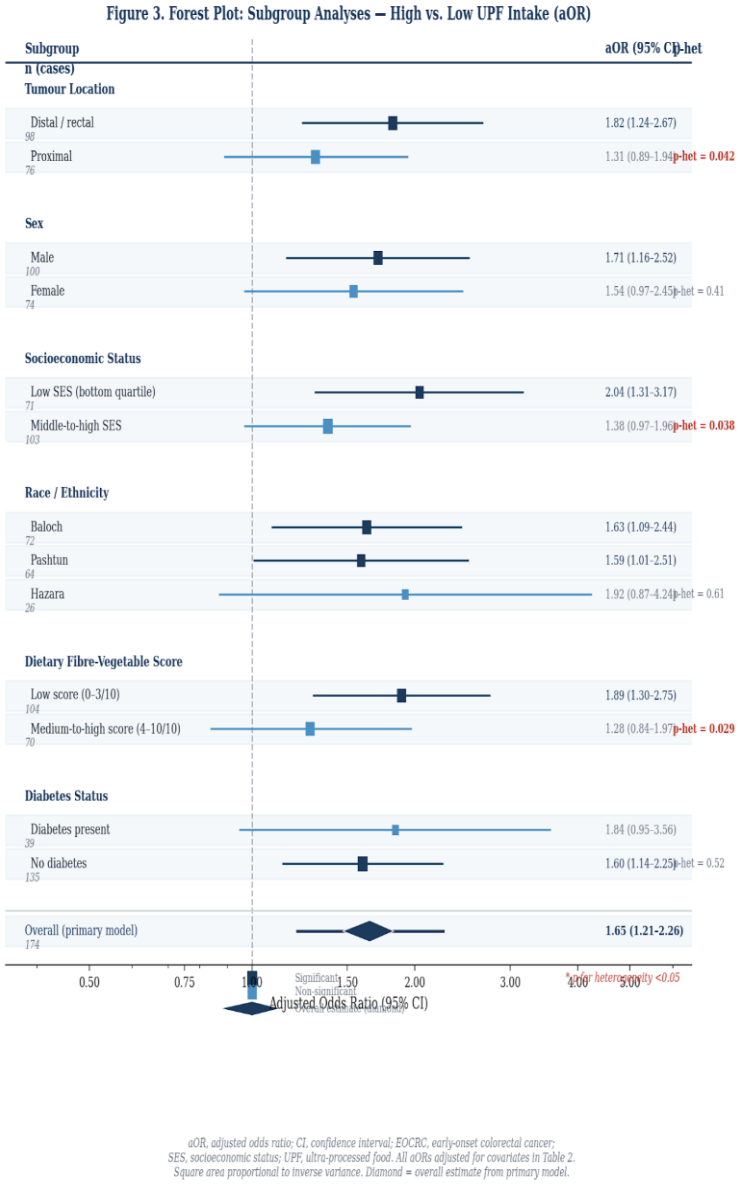

Figure 3. Forest plot of adjusted odds ratios (aORs) for high UPF intake (top vs. bottom tertile) across pre-specified subgroups. Filled squares represent point estimates (area proportional to inverse variance); horizontal lines indicate 95% CIs. Diamond indicates the overall estimate from the primary model. Dashed vertical line: null (OR=1.0). Statistically significant p values for heterogeneity ($p<0.05$) are highlighted in red. aOR, adjusted odds ratio; CI, confidence interval; SES, socioeconomic status; UPF, ultra-processed food.

DISCUSSION: This retrospective matched case-control study provides, to our knowledge, the first evidence from a resource-limited South Asian setting examining the joint contributions of UPF intake, cumulative antibiotic exposure, and systemic inflammation to early-onset colorectal cancer risk. Our primary findings—that high UPF intake, cumulative antibiotic exposure ≥3 courses, and elevated hsCRP each independently increase EOCRC odds, with significant multiplicative and additive synergy between the first two—are internally consistent with and extend the emerging literature from high-income cohorts.

The adjusted OR of 1.65 for high UPF intake falls within the range of effect sizes reported in the broader literature, and slightly exceeds the 45% adenoma risk increase reported from the Nurses' Health Study II in 2025.10 The higher estimate in our analysis may reflect the more clinically severe outcome measured (invasive adenocarcinoma rather than adenoma), differences in UPF product composition in the Pakistani market—notably higher trans-fat and synthetic additive content in informally manufactured snacks—or residual unmeasured confounding. The dose-response beginning at approximately 6 servings/day suggests that risk accrues at relatively modest consumption levels, with important implications for dietary counselling thresholds in clinical practice.

The association between cumulative antibiotic exposure and EOCRC (aOR 1.47 for ≥3 courses) is consistent with evidence from large observational studies reporting ORs of approximately 1.3–1.5 for multiple antibiotic prescriptions versus none.12,13 The finding that broad-spectrum agents carry slightly higher risk than narrow-spectrum agents aligns mechanistically with the broader microbiome-depleting spectrum of fluoroquinolones and cephalosporins. Critically, the interaction finding—that combined high UPF intake and ≥3 antibiotic courses yield a joint aOR of 2.98 with significant multiplicative and additive interaction—has not been previously reported, to our knowledge. A biologically plausible mechanism exists: dietary emulsifiers (polysorbate-80, carboxymethylcellulose) degrade the intestinal mucus layer and deplete butyrate producers such as Faecalibacterium prausnitzii,20 while broad-spectrum antibiotics independently deplete the same protective taxa. The convergent dysbiotic effect of both exposures may accelerate the transition from normal mucosa to adenoma and invasive carcinoma.

The mediation analysis finding that hsCRP elevation accounts for approximately one-quarter of the UPF–EOCRC association is consistent with mechanistic evidence that dietary emulsifiers and advanced glycation end-products activate the NLRP3 inflammasome and NF-κB pathway, elevating circulating interleukin-6 and downstream CRP.21 The substantial direct effect (75% of total) remaining after removing the hsCRP-mediated component confirms that circulating inflammation biomarkers do not fully capture the mechanisms linking UPF to CRC risk; complementary pathways including genotoxic effects of nitrites, disrupted bile acid profiles, and reduced butyrate-mediated colonocyte autophagy are likely contributors.

The anatomical specificity—stronger associations for distal and rectal tumours (aOR 1.82) than proximal tumours (aOR 1.31)—is consistent with international incidence data demonstrating that the rising EOCRC trend is predominantly driven by left-sided and rectal cancers, which more frequently exhibit chromosomal instability rather than microsatellite instability pathways.22 This pattern supports a luminal or microbiome-mediated mechanism,

given that bacterial density and fermentation are greatest in the distal colon and rectum.

The amplification of the UPF–EOCRC association in the lowest SES quartile (aOR 2.04) warrants particular attention in the Pakistani context. This likely reflects the confluence of higher dependence on low-cost energy-dense UPF, greater informal antibiotic use without prescription—well documented in Pakistan and disproportionately affecting lower-income households16—and reduced access to protective factors including dietary fibre, regular physical activity, and aspirin use. These findings strengthen the case for targeting EOCRC prevention at socioeconomically disadvantaged young adults through subsidised dietary counselling, enforced antibiotic stewardship in the informal pharmacy sector, and risk-stratified earlier screening.

Strengths and Limitations: Strengths include: objective ascertainment of antibiotic exposure from pharmacy records; histological confirmation of all case diagnoses; matching on five confounders; pre-registered analysis plan; mediation analysis quantifying the inflammatory pathway; E-value sensitivity analysis; and full STROBE compliance. The inclusion of both multiplicative and additive interaction analyses strengthens the synergy findings.

Limitations include: retrospective dietary assessment with potential differential recall bias (cases may over-report past UPF intake); informative missingness of fecal calprotectin (ordered in only 27.2% of participants); hereditary syndrome exclusion based on family history rather than germline sequencing; single-centre design limiting generalisability; cross-sectional ascertainment of inflammation markers (elevated CRP may partly reflect subclinical tumour-driven inflammation, limiting causal inference for mediation); and residual confounding by unmeasured exposures (E-value 2.74 establishes a reasonably high bar for such confounding to fully explain primary findings).

CONCLUSIONS:

In this retrospective matched case-control study, high ultra-processed food intake and cumulative antibiotic exposure were each independently associated with early-onset colorectal cancer risk at a tertiary referral centre in Quetta, Pakistan, with a statistically significant synergistic interaction and approximately one-quarter of the dietary association mediated through systemic inflammation. If confirmed in a prospective multicentre study with objectively measured dietary data and gut microbiome profiling, these findings would provide Level 2 evidence for three modifiable risk factors in an underrepresented population with a rapidly rising EOCRC burden. Immediate translational implications include evidence-based dietary counselling thresholds for UPF in young adults, antibiotic stewardship policies targeting informal pharmacy dispensing in lower-income communities, and risk-stratified screening beginning at age 40 for young adults with multiple identified risk factors.

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Author's Contribution:

JA: Conceived and designed the study, involved in data collection, performed statistical analysis and writing the manuscript.
BuR, FU, AS: Collected the data, critical review and preparation of manuscript.

All authors have read, approved the final manuscript and are responsible for the integrity of the study.
Data Availability: De-identified data supporting these findings are available from the corresponding author upon reasonable request.

REFERENCES:

1. Siegel RL, Jakubowski CD, Fedewa SA, Davis A, Azad NS. Colorectal cancer in the young: epidemiology, prevention, management. Am Soc Clin Oncol Educ Book. 2020;40:1–14.
2. Vuik FE, Nieuwenburg SA, Bardou M, Lamarque D, Neefjes-Borst A, van Ballegooijen M, et al. Increasing incidence of colorectal cancer in young adults in Europe over the last 25 years. Gut. 2019;68(10):1820–6.
3. Akimoto N, Ugai T, Zhong R, Hamada T, Fujiyoshi K, Ogata N, et al. Rising incidence of early-onset colorectal cancer: a call to action. Nat Rev Clin Oncol. 2021;18(4):230–43.
4. Khokhar N, Gill ML. Colorectal cancer in Pakistan: a single centre 10-year experience. J Coll Physicians Surg Pak. 2008;18(3):147–50.
5. Awan S, Shah SA, Shoukat A. Early-onset colorectal cancer in Pakistan: clinical and pathological characteristics. J Pak Med Assoc. 2022;72(5):893–7.
6. Stoffel EM, Murphy CC. Epidemiology and mechanisms of the increasing incidence of colon and rectal cancers in young adults. Gastroenterology. 2020;158(2):341–53.
7. Monteiro CA, Cannon G, Levy RB, Moubarac JC, Louzada MLC, Rauber F, et al. Ultra-processed foods: what they are and how to identify them. Public Health Nutr. 2019;22(5):936–41.
8. Fiolet T, Srour B, Sellem L, Kesse-Guyot E, Allès B, Méjean C, et al. Consumption of ultra-processed foods and cancer risk: results from the NutriNet-Santé prospective cohort. BMJ. 2018;360:k322.

9. Lerner A, Matthias T. Changes in intestinal tight junction permeability associated with industrial food additives explain the rising incidence of autoimmune disease. Autoimmun Rev. 2015;14(6):479–89.
10. Wang C, Du M, Kim H, Nguyen LH, Wang QL, Drew DA, et al. Ultra-processed food consumption and risk of early-onset colorectal cancer precursors among women. JAMA Oncol. 2026;12(1):49–57. doi:10.1001/jamaoncol.2025.4777.
11. Baxter NT, Schmidt AW, Venkataraman A, Kim KS, Waldron C, Martens EC. Dynamics of human gut microbiota and short-chain fatty acids in response to dietary interventions with three fermentable fibers. mBio. 2019;10(1):e02566-18.
12. Dik VK, van Oijen MG, Smeets HM, Siersema PD. Frequent use of antibiotics is associated with colorectal cancer risk: results of a nested case-control study. Dig Dis Sci. 2016;61(1):255–64.
13. Cao Y, Wu K, Mehta R, Drew DA, Song M, Lochhead P, et al. Long-term use of antibiotics and risk of colorectal adenoma. Gut. 2018;67(4):672–8.
14. Aleksandrova K, Boeing H, Nöthlings U, Jenab M, Fedirko V, Kaaks R, et al. Inflammatory and metabolic biomarkers and risk of liver and biliary tract cancer. Hepatology. 2014;60(3):858–71.
15. Nimptsch K, Aleksandrova K, Boeing H, Janke J, Lee YA, Launer L, et al. Association of CRP genetic variants with colorectal cancer risk. Int J Cancer. 2015;136(5):1181–91.
16. Van Boeckel TP, Gandra S, Ashok A, Caudron Q, Grenfell BT, Levin SA, et al. Global antibiotic consumption 2000 to 2010: an analysis of national pharmaceutical sales data. Lancet Infect Dis. 2014;14(8):742–50.
17. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP, et al. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. Lancet. 2007;370(9596):1453–7.
18. Valeri L, VanderWeele TJ. Mediation analysis allowing for exposure-mediator interactions and causal interpretation: theoretical assumptions and implementation with SAS and SPSS macros. Psychol Methods. 2013;18(2):137–50.
19. VanderWeele TJ, Ding P. Sensitivity analysis in observational research: introducing the E-value. Ann Intern Med. 2017;167(4):268–74.
20. Chassaing B, Koren O, Goodrich JK, Poole AC, Srinivasan S, Ley RE, et al. Dietary emulsifiers impact the mouse gut microbiota promoting colitis and metabolic syndrome. Nature. 2015;519(7541):92–6.
21. Chen J, Zhao KN, Vitetta L. Effects of intestinal microbial elaborated butyrate on oncogenic signaling pathways. Nutrients. 2019;11(5):1026.
22. Holowatyj AN, Lieu CH, Lewis MA, Ose J, Khoury J, Geloian M, et al. Racial/ethnic patterns of young-onset colorectal cancer. J Natl Cancer Inst. 2016;108(8):djw099.
23. Pearlman R, Frankel WL, Swanson B, Zhao W, Yilmaz A, Miller K, et al. Prevalence and spectrum of germline cancer susceptibility gene mutations among patients with early-onset colorectal cancer. JAMA Oncol. 2017;3(4):464–71.
24. Song M, Chan AT. Environmental factors, gut microbiota, and colorectal cancer prevention. Clin Gastroenterol Hepatol. 2019;17(2):275–89.
25. Xi Y, Xu P. Global colorectal cancer burden: trends, risk factors and prevention. Cancer Lett. 2021;521:255–64.
26. Mehta RS, Song M, Nishihara R, Drew DA, Wu K, Qian ZR, et al. Dietary patterns and risk of colorectal cancer: analysis by tumor location and molecular subtypes. Gastroenterology. 2017;152(8):1944–53.
27. Versporten A, Zarb P, Caniaux I, Gros MF, Drapier N, Miller M, et al. Antimicrobial consumption and resistance in adult hospital inpatients in 53 countries: results of an internet-based global point prevalence survey. Lancet Infect Dis. 2018;18(11):1211–23.
28. Kim SE, Paik HY, Yoon H, Lee JE, Kim N, Sung MK. Sex- and gender-specific disparities in colorectal cancer risk. World J Gastroenterol. 2015;21(17):5167–75.
29. Canani RB, Costanzo MD, Leone L, Pedata M, Meli R, Calignano A. Potential beneficial effects of butyrate in intestinal and extraintestinal diseases. World J Gastroenterol. 2011;17(12):1519–28.
30. Simin J, Fornés R, Liu Q, Czene K, Stec M, Ye W, et al. Antibiotic use and risk of colorectal cancer: a systematic review and dose-response meta-analysis. Br J Cancer. 2020;123(12):1825–32.