Diet and risk of oral potentially malignant disorders in rural Sri Lanka

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BACKGROUND: While protective role of antioxidant nutrients against cancer is well established, data on Asian diets in patients with oral cancer are meagre.

METHODS: A total of 1029 subjects over 30 years of age were investigated on their dietary practices in the Sabaragamuwa province in 2006–07. Data collection tools were an interviewer-administered questionnaire, a three-day food diary and an examination of the oral cavity. Subjects identified with Oral Potentially Malignant Disorders (OPMD) and disease-free controls were analysed in a case–control fashion. Among the OPMDs, those with leukoplakia were separately considered. A further subgroup analysis was undertaken for β -carotene-rich foods. The analysis was stratified by portions of fruit/vegetables consumed as five or more portions and two or more portions daily.

RESULTS: A low BMI (<18.5) was a significant independent risk factor for the development of OPMD. More than half of both cases and controls consumed less than two portions of fruit/vegetables per day and only 20 subjects consumed more than five portions per day. Intake of more than two portions per day of β -carotene-containing fruits/vegetables significantly reduced the risk of having an OPMD and leukoplakia (OR = 0.5; 95% CI, 0.3–0.9). The significant differences observed with BMI and fruits/vegetables were attenuated when adjusted for betel quid chewing, smoking and alcohol use.

CONCLUSIONS: This study discloses prevailing under-nutrition in this rural population with very low daily consumption of fruit/vegetables. Cancer preventive properties in their diets are limited and are swamped by the known carcinogenic agents associated with use of betel quid, tobacco and alcohol.

Keywords: BMI; fruit and vegetables; oral cancer; oral potentially malignant disorders; risk factors; Sri Lanka; β -carotene

Introduction

Squamous cell carcinoma of the oral cavity is often preceded by oral potentially malignant disorders (OPMD), the term recommended by the WHO Collaborating Centre for Oral Cancer/Precancer to encompass what were earlier referred to as 'precancer' or 'pre-malignant lesions and conditions' (1). In this study, we considered oral leukoplakia, oral submucous fibrosis (OSF), erythroplakia and lichen planus as part of the spectrum of OPMD. The global prevalence of OPMD has been reported at between 1 and 5% (2), but higher prevalences are described from South and South-East Asia, with male preponderance: for example, Taiwan (12.7%) (3); and in some Western Pacific countries, for example, Papua New Guinea, 11.7% (4). Such wide geographical variations are due to lifestyles specific to the country or region. In a screening programme undertaken in Sri Lanka, the prevalence of oral precancer in the 1980s was reported as 4.2% (5).

Dietary factors are estimated to account for approximately 30% of cancers in Western countries (6). This proportion is currently thought to be about 20% in developing countries and is projected to increase in the future (7). Studies have revealed that a diet low in fresh fruits and vegetables is a significant risk factor for oral cancer in young subjects in the UK (8) and for OPMD in Japanese subjects living in the UK (9). A case–control study undertaken, with data from an ongoing randomized oral cancer screening trial in Kerala, India yielded an inverse dose–response relationship between BMI and risk of leukoplakia (P for trend = 0.007) (10).

Evidence for the bio-protective effect of the diet comes from case–control and cohort studies, from animal and from *in vitro* experiments. The micronutrients in food which confer these benefits are also well understood. Vitamin A and related carotenoids (in particular β -carotene), vitamin C

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and selenium appear to be particularly protective against most epithelial cancers and their precursor lesions (11–14), and much of the effect is attributable to their antioxidant activities. Antioxidants act by reducing free radical reactions that can cause DNA mutations and changes in lipid peroxidation of cellular membranes (15, 16). Other protective roles of micronutrients are modulation of carcinogen metabolism, maintenance of appropriate cell differentiation, inhibition of cell proliferation and oncogene expression, maintenance of immune function and inhibition of formation of endogenous carcinogens (17).

A recent meta-analysis on risk factors for oral cancer, based on 15 case–control studies and one cohort study, was able to utilize diet data from nearly 5000 subjects: this estimated that each portion of fruit or vegetables consumed per day reduced the risk of oral cancer by around 50% (18). These effects are also demonstrable with OPMD: in a population-based case–control study in Japan, serum levels of lycopene and beta-carotene were significantly lower in those with leukoplakia; logistic regression of their data showed that high levels of β -carotene were related to low risk of oral leukoplakia (OR = 0.16% CI: 0.03–0.86) (19).

Intervention studies are also encouraging in this respect. In a double-blind placebo controlled trial in Kerala, India (14), up to one-third of subjects showed regression of their oral leukoplakias after 12 months supplementation with oral β -carotene. An open-ended trial with multiple micronutrient supplementation over one to 3 years improved the symptomatology of 117 oral submucous fibrosis (OSF) patients in Pakistan (20).

Tea has strong protective effects against many cancers. Most research has examined green tea (21–23), but black tea is also protective (24–27). It is predominantly the polyphenols present in tea that act as antioxidants to counteract both initiation and promotion of carcinogenesis (15).

The role of chillies in the diet in either promoting oral carcinogenesis or providing protection against oral cancer has not been examined in detail. The phytochemicals in spices, including chillies, have potent antioxidant and enzyme activities, which have the potential to reduce the risk of cancer through several complementary and overlapping mechanisms (28).

Due to the paucity of studies on dietary factors and OPMD in Sri Lanka, we investigated the association of OPMD (and leukoplakia as a subgroup) with consumption of fruits, vegetables, chillies and tea.

Materials and methods

A cross-sectional community survey, employing a house-to-house method to interview and perform a visual screening for OPMD, was conducted in the Sabaragamuwa province. A total of 1029 subjects were selected by a multistage, stratified and clustered sampling technique. We have described the study population in detail elsewhere: approximately 87% lived in small villages and were employed in farming; around 9% lived and worked on tea and rubber estates (29, 30). Ethical approval was obtained from the Faculty of Medicine, University of Colombo, and subjects signed for their informed consent before data collection.

An interviewer-administered questionnaire collected the socio-demographic variables of age, gender, ethnicity, occupation and level of education. Lifestyle questions covered betel quid chewing, smoking and use of alcoholic beverages, defined as those who had never, or ever, practised a habit. 'Ever' habits were further subdivided into past, occasional and weekly/daily (31, 32). Weight and height of subjects were measured to calculate BMI.

Consumption of fruit, vegetables and tea was measured with a three-day diet diary for 2 days in the week and 1 day on the weekend, prior to the oral examinations. Participants were instructed to write the items and amount consumed according to common household utensil units (e.g. 'tablespoonful') without changing their food consumption pattern.

From November 2006 to November 2007, the first author examined the oral cavities of all 1029 subjects above the age of 30 years, blinded to their risk factor status at the time of examination. The diagnostic criteria for the detection of OPMD: leukoplakia and erythroplakia were based on WHO criteria; and for OSF, lichen planus and other oral mucosal abnormalities, on criteria used for previous screening programmes (1, 33, 34). Oral verrucous hyperplasia although considered an entity in the OPMD group (particularly in Taiwan) is not encountered in Sri Lanka and is therefore not included in our study (3, 35). Chewer's mucosa, quid-induced lichenoid reactions, smoker's keratosis, denture stomatitis, angular cheilitis and pallor and depapillation of tongue were considered as 'other' oral mucosal abnormalities. Definitions of these conditions and descriptions of sampling and data collection methods are described elsewhere (29).

The subjects identified with OPMD during this cross-sectional study were analysed in a case–control fashion to assess strength of the association between diet and OPMD. The main subgroup of OPMDs, that is, oral leukoplakia cases was separately analysed. 'Controls' were selected from subjects free of any oral mucosal disorders at the time of screening.

Statistical analysis

Data were recorded on paper, using the pre-tested questionnaire (available on request from principal author), and entered into the SPSS 17 software package, which was used for all statistical analyses. The relationships between two categorical variables were tested by chi-square. Correspondence analysis was used to combine information on occupation and education to produce a single score for SES, on a continuous scale, for each subject (36). Consumed fruit and vegetables were later quantified into portions (37): 3–4 spoonfuls of cooked vegetable, 10 spoonfuls of uncooked/raw vegetable, one medium-sized banana, orange and apple, 9–11 grapes or similar fruits, one-fourth of medium size pawpaw, a one half avocado and 100 ml cup of undiluted fruit juice were considered as single portions (Fig. 1). A tea cup was defined as the 100 ml vessel shown in Fig. 1.

The fruits and vegetables consumed were then classified based on national guidelines according to the content of β -carotene in each item. Food items that do not contain beta-carotene, according to the Hector Kobbakaduwa Agriculture Research Centre, were counted separately (38).

Figure 1 Portion sizes of different fruit items.

Once food items were identified, the weighted daily average intake of each category of food portions consumed was calculated using the following formula (39):

$$\text{Average intake of fruit and vegetable portion} = \frac{[(5 \times 2 \text{ working days}/2) \pm (\text{Saturday or Sunday}) \times 2]}{7}$$

The strength of association between a given food and clinical disease was tested in a case-control fashion. Bivariate analysis was carried out to assess the strength of the association, and a logistic regression model was used to define the effects of controlling potentially confounding variables. All other potential confounding variables were included as covariates. Of these, age, BMI- and β -carotene-containing vegetable and fruit portions were entered as continuous variables, the remainder as categorical variables. An enter-method conditional approach was used with p-values less than 0.05 considered statistically significant.

A subgroup analysis was carried out separately to explore the protective effect of fruit and vegetables for leukoplakia as a dependent variable and collectively as OPMD. This analysis took into account two portions and five portions of fruit and vegetables consumed per day as cut-off values.

Results

Among the 1029 subjects screened, 102 cases of OPMD were detected. Among OPMD: leukoplakia was most common 71 (8.9%), with OSF second 25 (1.7%) and the remainder of cases were lichen planus 6 (0.7%). In addition, there were four cases of oral cancer, one newly diagnosed and three under treatment and one case of treated leukoplakia: these were excluded from the analysis. One hundred and ninety-five subjects diagnosed with other oral mucosal diseases were excluded, leaving 728 healthy subjects designated as controls for the case-control analysis. When weighted for age and place of residence – namely village or Estate – this represents a prevalence of 11.3% for OPMD (32). Of the 1029 subjects, 914 completed the food diary, a response rate of 89%.

In the present study, perusal of the food diaries revealed diets to be rich in starchy vegetables such as jack fruit, breadfruit and yams. Large quantities of these were consumed. More than 50% of both cases and controls consumed less than 2 portions of fruit and vegetables per day. The WHO recommended consuming 5 portions as a guideline, but in our study, only 20 subjects consumed more than 5 portions per day; 99% of the cases and 97% of the controls consumed less than 5 portions of fruit and vegetable per day.

Table 1 shows the association of OPMD with total vegetable and fruit portions consumed per day and with BMI. In 42% of the cases and 30.6% of the controls, the BMI was less than 18.5. Only 14.7% of the study subjects were in overweight and obese (BMI > 25) categories. ORs were reduced with normal weight (BMI 18.5–25) and overweight/obese categories (BMI > 25) compared with those of BMI < 8.5. This was not significant when adjusted for other confounding factors: betel quid chewing, smoking, alcohol use and socio-demographic factors such as age, sex, education and occupation. As shown in Table 1, no association was observed for consumption of total vegetable and fruit portions per day at either 2 portions and 5 portions as cut-off values.

Intake of β -carotene-containing vegetables and fruit portions and risk of occurrence of OPMD was not statistically significant when considered as 5 portions of fruits and vegetables. The combined effect of consuming more than 2 portions per day of β -carotene-containing vegetables plus fruit portions have shown crude OR of 0.5; 95% CI, 0.3–0.9. However, the logistic regression analysis shows an attenuation of the protective effect of beta-carotene-rich vegetables and fruits when controlling for other covariates, the adjusted OR being 0.8 (CI 0.4–1.4) (Table 1).

Table 2 shows the risk of occurrence of leukoplakia with total vegetable and fruit portions consumed per day and with BMI. In 37% of the cases and 30.6% of the controls, the BMI was less than 18.5. Only 15% of the study subjects were in overweight and obese (BMI > 25) categories. ORs were reduced with normal weight (BMI 18.5–25) and overweight/obese categories (BMI > 25) compared with those of BMI < 18.5, but it was not significant.

No association was observed for risk of occurrence of leukoplakia and consumption of total vegetable and fruit portions per day when separately considering either two portions or five portions as cut-off values.

Intake of β -carotene-containing vegetables and fruit portions and risk of occurrence of leukoplakia was not statistically significant in the group consuming 5 portions of fruits and vegetables compared with the rest. The combined effect of consuming more than 2 portions per day of β -carotene-containing vegetables plus fruit portions have shown crude OR of 0.5; 95% CI, 0.3–0.9. However, on logistic regression analysis an attenuation of the protective effect of beta-carotene-rich vegetables and fruits was noted when controlling for other covariates, the adjusted OR being 0.8 (CI 0.4–1.7) (Table 2).

Table 3 illustrates the association between OPMD and the number of cups of tea consumed per day, and with chillie consumption. More than two cups of tea were

Table 1 Risk of occurrence of OPMD and BMI, consumption of total fruits/Vegetables and β -carotene containing fruit/vegetable portions per day

Characteristics	OPMD Cases (n = 101) n (%)	Controls (n = 728) n (%)	P-value	Crude OR ($\pm$ 95% CI)	Adjusted ^a OR ($\pm$ 95% CI)
BMI					
0–18.5	42 (41.6)	221 (30.6)		1.0	1.0
>18.5–25	50 (49.5)	389 (53.9)	0.08	0.7 (0.4–1.0)	0.8 (0.5–1.3)
>25	9 (8.9)	112 (15.5)	0.02	0.4 (0.2–0.9)	0.8 (0.4–1.9)
Missing		6			
Total fruit and vegetable portions per day					
<5 portions	94 (98.9)	622 (97.0)		1.0	
>5 portions	1 (1.1)	19 (3.0)	0.307	0.3 (0.0–2.6)	1.4 (0.7–2.6)
Missing	6	87			
Total fruit and vegetable portions per day					
<2 portion	61 (64.2)	335 (52.3)		1.0	1.0
>2 portions	34 (35.8)	306 (47.7)	0.03	0.6 (0.4–0.9)	1.1 (0.6–1.8)
Missing	6	87			
β -carotene containing fruit and vegetable portions per day					
<5 portion	95 (100.0)	628 (98.0)		1.0	1.0
>5 portions	0	13 (2)	0.30	0.5 (0.0–3.9)	0.000
Missing	6	87			
β -carotene containing fruit and vegetable portion per day					
<2 portion	75 (78.9)	424 (66.1)		1.0	1.0
>2 portions	20 (21.1)	217 (33.9)	0.01	0.5 (0.3–0.8)	0.8 (0.4–1.4)
Missing	6	87			
Total	101 (100.0)	728 (100.0)			

^aOR adjusted for sex, age, occupation, education, BMI, smoking, betel chewing and alcohol drinking.

Table 2 Risk of occurrence of leukoplakia and BMI, consumption of total fruits/Vegetables and β -carotene containing fruit/vegetable portions per day

Characteristics	Leukoplakia (n = 70) n (%)	Controls (n = 728) n (%)	P-value	Crude OR ($\pm$ 95% CI)	Adjusted ^a OR ($\pm$ 95% CI)
BMI					
0–18.5	26 (37.1)	221 (30.6)		1.0	1.0
>18.5–25	36 (54.1)	389 (53.9)	0.37	0.7 (0.4–1.3)	1.0 (0.6–1.9)
>25	8 (11.4)	112 (15.5)	0.23	0.6 (0.2–1.4)	1.4 (0.6–3.6)
Total fruit and vegetable portion per day					
<5 portions	65 (98.5)	622 (97.0)		1.0	
>5 portions	1 (1.5)	19 (3.0)	0.507	0.5 (0.0–3.8)	0.8 (0.0–8.2)
Missing	4	87			
Total fruit and vegetable portions per day					
<2 portion	42 (63.6)	335 (52.3)		1.0	1.0
>2 portions	24 (36.4)	306 (47.7)	0.08	0.6 (0.4–1.0)	1.1 (0.6–2.1)
Missing	4	87			
β -carotene containing fruit and vegetable portions/day					
<5 portions	66 (100.0)	628 (98.0)		1.0	1.0
>5 portions	0	13 (2)	0.76	0.7 (0.1–5.6)	0.000
Missing	4	87			
β -carotene containing fruit and vegetable portions/day					
<2 portion	52 (78.8)	424 (66.1)		1.0	1.0
>2 portions	14 (21.2)	217 (33.9)	0.04	0.5 (0.3–0.9)	0.8 (0.4–1.7)
Missing	4	87			
Total	70 (100.0)	728 (100.0)			

^aOR adjusted for sex, age, occupation, education, BMI, smoking, betel chewing and alcohol drinking.

consumed per day by 30% of the cases, and by 28% of the controls: these differences were not statistically significant. Twenty-four per cent of the cases consumed high amounts of chillie and 66% of cases consumed medium amounts of chillie with meals. The controls reported similar patterns of chillie consumption.

Table 4 shows a statistically significant association between alcohol drinking and low consumption of fruits and vegetables. The majority (80%) of weekly drinkers were

consuming less than two portions of 'beta-carotene-high vegetable and fruit portions' per day. Perusal of the food diaries of chronic heavy alcohol users revealed that most frequently missed dinner altogether. Half of the regular alcohol drinkers were taking only one cup of tea per day.

The final logistic regression model has shown, betel quid chewing and alcohol consumption are the only statistically significant characteristics remained after controlling the other factors which were already published in 2009 (30).

Table 3 Association between OPMD and consumption pattern of tea and chillies

Characteristics	OPMD Cases (n = 101) n (%)	Controls (n = 728) n (%)	P-value	Crude OR (± 95% CI)	Adjusted ^a OR (± 95% CI)
Consumption of tea, cups					
≤ 1 per day	36 (37.9)	252 (39.9)		1.0	1.0
>1 to ≤ 2 per day	30 (31.6)	203 (32.1)	0.898	1.0 (0.6–1.7)	1.4 (0.8–2.4)
>2 per day	29 (30.5)	177 (28.0)	0.609	1.1 (0.7–1.9)	1.3 (0.7–2.4)
Missing	6	96			
Temperature of tea consumption					
Lukewarm	12 (12.0)	57 (7.9)		1.0	1.0
Mildly hot	68 (68.0)	520 (72.1)	0.165	0.6 (0.3–1.2)	0.8 (0.4–1.6)
Hot	20 (20.0)	144 (20.0)	0.295	0.6 (0.3–1.4)	0.8 (0.3–1.8)
Missing	1	7			
Chillie consumption					
Low	10 (10.0)	38 (5.2)		1.0	1.0
Medium	66 (66.0)	506 (69.9)	0.064	0.5 (0.2–1.0)	0.7 (0.3–1.4)
High	24 (24.0)	180 (24.9)	0.103	0.5 (0.2–1.1)	0.6 (0.4–1.5)
Missing	1	4			
Total	101 (100.0)	728 (100.0)			

^aOR adjusted for sex, age, occupation, education, BMI, smoking, betel chewing and alcohol drinking.

Use of betel quid shows a strong dose–response relationship. The adjusted OR for daily chewers was 10.6 (95% CI, 3.6–31.0) after controlling for all other variables.

Regular alcohol drinking was associated with an increased risk of OPMD, the adjusted OR for weekly drinkers being 3.5 (1.6–8.0).

Discussion

In the published literature, there is convincing evidence that high intake of fruit and vegetables decreases the risk of cancers of the mouth and pharynx, oesophagus, lung, stomach, colon and rectum (40, 41) and their precursor lesions (20, 42). Micronutrients such as vitamins A, C, E and carotenoids (in particular β-carotene and lycopene)

have important protective effects, and selenium plays an important role in reducing the incidence of oral leukoplakia (9, 14). It was reported in Japan (19) that a high level of beta-carotene was associated with a low risk of oral leukoplakia. Beta-carotene-rich vegetables such as broccoli, carrots and peppers (capsicums) or green leafy vegetables appear to provide greater protection than vegetables lacking β-carotene.

In the present study, as most subjects consumed large quantities of starchy vegetables, it was decided to segregate β-carotene-rich vegetables and fruits as a subgroup. Statistically significant protective effects for occurrence of OPMD and leukoplakia were observed with high intakes of β-carotene-containing fruits and vegetables, with a crude OR of 0.50. However, this protective effect was attenuated when adjusted for confounding factors: the protective effects of certain foods may not be exhibited in a population in which more than one-third of subjects are under-nourished. Consistent with this, bivariate analysis indicated that low BMI (<18.5) was associated with increased risk of OPMD. Similar results have been reported in the pooled analysis of 15 case–control studies from the INHANCE consortium: low BMI, smoking and alcohol consumption increase ORs for head and neck cancer and ORs are reduced in healthy weight, overweight and obese categories (43). Study conducted in Trivandrum city, Kerala, India also reported similar results for BMI and risk of leukoplakia (10).

Based on the evidence that antioxidants in the diet may provide protection against oral cancer, extensive studies from the MD Anderson Cancer Centre in the USA are progressively identifying the most effective combinations of antioxidant supplements of value in promoting the regression of OPMD and the prevention of recurrences and second primary neoplasms in subjects with head and neck cancer. It has to be recognized, however, that these agents do not always prevent the progression of an OPMD to overt cancer (44, 45).

Green tea has been shown to reduce oral cancer risk by close to 60% (for those drinking > 5 cups/day) and 35%

Table 4 Distribution of subjects according to weekly consumption of alcohol

Characteristics	Alcohol consumers			P-value
	Never n (%)	Weekly n (%)	Total n (%)	
β-carotene containing vegetable portions per day				
≤ 1 portion	153 (28.8)	35 (42.2)	188 (30.6)	0.006 ($\chi^2_2 = 10.3$)
>1 to ≤ 2 portions	187 (35.2)	32 (38.6)	219 (35.7)	
>2 portions	191 (36.0)	16 (19.3)	207 (33.7)	
Missing	59	15	74	
Total fruit & vegetable portions per day				
≤ 1 portion	96 (18.1)	30 (36.1)	126 (20.5)	<0.001 ($\chi^2_2 = 10.3$)
>1 to ≤ 2 portions	162 (30.5)	30 (36.1)	192 (31.3)	
>2 portions	273 (51.4)	23 (27.7)	296 (48.2)	
Missing	59	15	74	
Consumption of tea: cups per day				
≤ 1 per day	198 (37.9)	40 (48.8)	238 (39.3)	0.154 ($\chi^2_2 = 10.3$)
>1 to ≤ 2 per day	173 (33.1)	24 (29.3)	197 (32.6)	
>2 per day	152 (29.1)	18 (22.0)	170 (28.1)	
Missing	67	16	83	
Total	590 (100.0)	98 (100.0)	688 (100.0)	

(for those drinking 1-2 cup/day) (46). Further, a recent Phase II trial of green tea supplements promoted regression of OPMD (47, 48). Historically, Sri Lankan populations have consumed large quantities of, predominantly black, tea, but this habit is now decreasing. In the present study, the number of cups of tea consumed per day was small, so it is not surprising that no significant protective effects could be demonstrated. The protective effect of tea could also be masked by the prevailing under-nourishment in the population under study here, and the quality of the tea may be poor in this predominantly low socioeconomic population. Cheap, adulterated tea is commonly found in local markets. The present study shows that alcohol drinkers consumed fewer cups of tea and fewer portions of fruit and vegetables than teetotallers, and tended to frequently miss dinner, compounding their risks of disease.

Studies of this nature are often subjected to information bias as the measurement of consumption of fruit and vegetables was based on self-reported data. People tend alter their food habits as it is requested. This could have been overcome by the biological assessment, which is limited applicability in the community based study.

A recent study conducted in Italy has shown that diets rich in animal products were associated with an increased risk of oral/pharyngeal cancers, and a protective effect was revealed with diets rich in starch, vitamin and fibre and unsaturated fat (49). The Authors concluded that the evidence for any association with diet and oral cancer remains weak and any clinical significance remains limited (49).

Consumption of fruits, vegetables and tea by these rural Sri Lankans is clearly inadequate. The WHO recommends 5 portions (400 g) of fruits and vegetables per day (15, 50). We recommend that this population be motivated by culturally sensitive educational programmes to grow and consume more home-grown fruits and vegetables, which could be produced relatively cheaply. Targeted health promotion messages on dietary guidelines on healthy eating to reduce risk of oral cancer need to be devised.

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of the business cycle, starting with the
on perfect competition, constant returns
production. In this environment goods
extra profits and the structure of market
he number of firms in the individual firm
etermined by the model. The New-Key
otaki (1987), has introduced product
general equilibrium models with nominal
ad and supply shocks. Most of this literat

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