

## Feasibility and Limitations of Acridine Orange Fluorescence Technique Using a Malaria Diagnosis Microscope in Myanmar

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We studied parasite detectability in thick films by an acridine orange fluorescence technique (AO) to test its applicability and the use of a Malaria Diagnosis Microscope (MDM)-ESL in the detection of parasites, compared to the conventional Giemsa staining method. This study was conducted on 1,390 clinically suspected malaria cases of Thaton township, Myanmar. We found sensitivities of 82.8% for *Plasmodium falciparum* (*P. falciparum*) and 100% for *Plasmodium vivax* (*P. vivax*) and specificities of 97.1% for *P. falciparum* and 98.6% for *P. vivax*. AO had a higher sensitivity than Giemsa-stained films at low levels of parasitemia (<1,000/ $\mu$ l). AO showed lower sensitivity and higher specificity than the Giemsa method at parasite levels of more than 1,000/ $\mu$ l. The results of using the AO method, achieved by both novice and experienced observers, showed no significant difference and required less practice to perform the test as well as to identify the parasite. The acridine orange fluorescence technique using a malaria diagnosis microscope MDM-ESL series is simple, rapid and cost effective. The microscope is conveniently operable using standard AC power or a 12-V DC car battery, and it is easily convertible to a conventional biological microscope. With the exception of species differentiation, which is not possible with this method, this method would be appropriate for both clinical and epidemiological studies.

**Key Words:** Malaria Diagnosis, acridine orange, fluorescence, *Plasmodium*

A prompt and accurate diagnosis is the key to effective disease management of malaria. In both clinical and epidemiological studies, diagnosis of malaria infection conventionally depends on the microscopical examination of blood films stained with Giemsa or the Field rapid stain method. Diagnosis accuracy depends on the experience of the observer, the quality of the blood smear prepared and the time given for the examination. A

simple and rapid method with high sensitivity is needed that could be used in all areas where malaria is a problem. Recent techniques using DNA or RNA probes have high sensitivity and high specificity, and have been tried for the rapid detection of malaria parasites especially of low level, but high cost and difficulty of application in the field are major obstacles to their use. One of the promising methods appears to be fluorochrome staining with acridine orange (AO) dye [1, 2]. With this method, there is a differential staining of nuclear DNA (yellow-green) and cytoplasm (red), which reveals the clear identification of the malaria parasite, but the need for an expensive

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## Camptothecin Induces Urokinase-type Plasminogen Activator Gene-Expression in Human RC-K8 Malignant Lymphoma and H69 Small Cell Lung Cancer Cells

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We previously reported that anthracyclines, which could generate reactive oxygen species (ROS), could induce the urokinase-type plasminogen activator (uPA) gene expression in human RC-K8 malignant lymphoma cells and in H69 small cell lung cancer (SCLC) cells. In screening other uPA-inducible anti-cancer agents, we found that camptothecin (CPT) and its derivative, SN38, could induce uPA in RC-K8 and H69 cells. CPT and SN38, which are also used for the treatment of lymphoma and SCLC, significantly increased the uPA accumulation in the conditioned media of both cells in a dose-dependent manner. The maximum induction of uPA mRNA levels was observed 24 h after stimulation. Pretreatment with pyrrolidine dithiocarbamate (PDTC), an anti-oxidant, inhibited the CPT-induced uPA mRNA expression. Thus, CPT induces uPA through gene expression, and, therefore, CPT may influence the tumor-cell biology by up-regulating the uPA/plasmin system.

**Key words:** CPT, SN38, uPA, RC-K8, H69

Urokinase-type plasminogen activator (uPA) catalyzes the conversion of zymogen plasminogen to plasmin and plays a central role in the fibrinolytic process. uPA is also related to tumor progression, and high levels of uPA suggest a poor prognosis of the cancer [1]. uPA is suggested to be one of the acute phase reactants whose synthesis is regulated by pro-inflammatory cytokines, such as tumor necrosis factor- $\alpha$  (TNF $\alpha$ ) and IL-1. These pro-inflammatory cytokines are also shown to generate reactive oxygen species (ROS) in human cells [2, 3]. Previously we demonstrated that IL-1 $\beta$  and lipopolysaccharide (LPS), along with hydrogen peroxide,

induced uPA gene expression in human malignant cells [4, 5]. Furthermore, anthracycline anti-cancer agents, which generate ROS, also induced uPA gene expression in both RC-K8 and H69 cells [6, 7]. ROS, such as super-oxide and hydrogen peroxide, work as signaling messengers, activating transcription factors such as NF- $\kappa$ B and AP-1, and inducing a number of gene expressions [8, 9]. NF- $\kappa$ B and AP-1 elements are also identified in the uPA promoter region [10], and, therefore, NF- $\kappa$ B and/or AP-1 transcription factors appear to be involved in the IL-1- and TNF $\alpha$ -induced-uPA expression [11, 12]

Similar to anthracyclines, CPT and its derivative SN38 also generate ROS [13, 14]. CPT is a cytotoxic agent that inhibits the nuclear enzyme Topoisomerase I (Topo I), and produces a complex with Topo I, also

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## Evaluation of a Method for Typing the Microsatellite D12S391 Locus Using a New Primer Pair and Capillary Electrophoresis

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We describe a modified method for typing a polymorphic microsatellite D12S391 locus by PCR using a newly designed primer pair. This primer pair produces shorter D12S391 amplified fragments (104-156 bp) than the primer pair originally described by Lareu *et al.* (209-261 bp). The detection system for the D12S391 locus using the new primer pair and capillary electrophoresis (CE) analysis was evaluated using various forensic samples. The typing results from 70 DNA samples using the new primer pair and the original primer pair were completely identical. One hundred twenty-five amplified fragments from D12S391 alleles were sized correctly within  $\pm 0.25$  bp of the D12S391 allelic ladder. A rare allele, 19.3, previously found only in Caucasians, was found for the first time in a Japanese subject, and it was clearly distinguished from allele 20 by the CE analysis. This detection system was sensitive and could detect D12S391 types from 16 pg of genomic DNA, and from a minor component at a ratio of 1:10 in mixed samples. This system was more useful for the analysis of degraded DNA than was the method using the original primer pair, and could detect D12S391 types from bloodstains that had been stored for 26 years. In addition, the specificity of the method was demonstrated using nonhuman DNA.

**Key words:** short tandem repeats, D12S391, forensic application, capillary electrophoresis

**F**or DNA typing from forensic biological evidence, in which the DNA is often damaged, the detection of short tandem repeats (STRs) using polymerase chain reaction (PCR) is effective, since the fragments to be amplified are short [1-4]. STR typing methods generally use product separation by slab gel electrophoresis with detection by silver staining or labeling with fluorescent dyes. Recently, capillary electrophoresis (CE) analysis, which provides rapidity, high resolution and precision, has been shown to be useful for the separation of amplified

fragments [5-7]. We previously reported the polymorphism of the D12S391 microsatellite locus and the allele frequency in a Japanese population sample [8]. This locus showed a compound STR consisting of a 4-base repetition based on (AGAT) $n$ (AGAC) $m$ (AGAT) $l$ . Fourteen different alleles, which were designated allele 15 to allele 28, ranging in size from 209 bp to 261 bp, were detected. In the present study, we modified the PCR reverse primer in order to obtain shorter amplified fragments (104-156 bp). In order to test this new primer pair in the examination of forensic biological evidence, we have evaluated it for accuracy, sensitivity, reproducibility, species specificity and for applications in mixed samples, degraded samples and aged bloodstains, using

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## Diabetic Gastroparesis in Association with Autonomic Neuropathy and Microvasculopathy

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Gastroparesis is a frequent and sometimes life-threatening complication of diabetes mellitus. Autonomic neuropathy seems to be one of the most important mechanisms underlying this entity, together with the other probable pathologies. The present study was performed in order to identify an alternative to gastric scintigraphy as a screening test. The gastric emptying times of 60 subjects (Group 1: 20 insulin-dependent patients, Group 2: 20 non-insulin-dependent diabetes mellitus patients, and Group 3: 20 healthy volunteers) were monitored by gastric scintigraphy. Perception thresholds for cold, heat, and vibration were tested by a quantitative sensory test, and QTc dispersions were calculated from standard electrocardiography recordings. In addition, fasting blood glucose, hemoglobin A<sub>1c</sub>, and urine  $\beta_2$ -microglobulin and microalbumin concentrations were determined for the patient groups. Funduscopy examination was performed by an independent ophthalmologist. Gastroparesis was determined in both patient groups, regardless of fasting blood glucose and hemoglobin A<sub>1c</sub> concentrations. A strong correlation was observed between nephropathy, retinopathy, and cardiac autonomic denervation (QTc) and gastroparesis. In conclusion, retinal and renal microvasculopathy parameters and cardiac autonomic function tests may be useful for screening diabetic patients for gastroparesis.

**Key words:** diabetic gastroparesis, microvasculopathy, autonomic neuropathy

**G**astroparesis is a frequent and sometimes life-threatening complication of diabetes mellitus (DM) [1-3]. Gastrointestinal motility abnormalities can cause nausea, vomiting, post-prandial fullness, early satiety, belching, and bloating. They also present a major problem for the regulation of blood glucose, especially in insulin dependent patients, because of the improper digestion and absorption of intake [4]. A consortium of pathological processes including hyperglycemia, gastrointestinal hormone changes, myogenic mechanisms, and

autonomic neuropathy are the causes of this entity [5-9]. Several studies have been conducted in order to identify a noninvasive method of evaluating patients for gastroparesis, such as a simple screening test similar to gastric scintigraphy [10-19]. Body mass index (BMI), fasting blood glucose, and hemoglobin A<sub>1c</sub> (HbA<sub>1c</sub>) values did not demonstrate a reliable correlation [20]. However, investigations concerning cardiovascular autonomic regulation, [21-22] vagal electrical activity, and mesenteric blood flow have shown striking correlations with gastroparesis [23-26].

In this study we investigated the relationship between gastroparesis and diabetic neuropathy, the components of neuropathic insult, and microvasculopathy. The aim of

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## Hepatopancreatic Arterial Ring: Bilateral Symmetric Typology in Human Celiaco-Mesenteric Arterial System

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The celiac and mesenteric arterial system including the left gastric, splenic, common hepatic, and superior mesenteric arteries shows various types of origins, courses, ramifications and anastomoses. In order to explain the various expressions of this system, we have proposed a typological model, in which celiacomesenteric arteries develop as paired or bilaterally symmetrical primordial vessels originated from the anterior aspect of the aorta, and these vessels anastomose each other with longitudinal and horizontal pathways. Here, we report 3 unusual cases characterized by arterial rings, formed by the left gastric, left accessory hepatic, proper hepatic, anterior pancreaticoduodenal, and dorsal pancreatic arteries. The dorsal pancreatic and anterior pancreaticoduodenal arteries are located to the right and left of the embryonic pancreas developing in the dorsal mesentery, respectively. Such hepatopancreatic arterial rings simultaneously containing right and left elements can only be explained using our typological model, in which the concept of paired arteries or bilateral symmetry is introduced.

**Key words:** arterial variation, celiac trunk, superior mesenteric artery, typology, bilateral symmetry

**T**hree unpaired arteries — the celiac trunk and the superior and inferior mesenteric arteries — usually originate from the anterior aspect of the abdominal aorta, and supply the abdominal digestive organs and spleen. Among these, the celiac trunk, a wide ventral branch just below the aortic hiatus, passes almost horizontally forward of and slightly above the pancreas and splenic vein, then dividing into the following: the left gastric, common hepatic, and splenic arteries. These three and the superior mesenteric arteries vary in terms of pattern of origins, courses, ramifications, and anas-

tomoses [1-14]. The celiac trunk or its branches may also give off one or both (left and right) the inferior phrenic arteries [15]. These variations have been classified and explained using classical typological models [16, 17]. The classical models are characterized by 4 longitudinally anastomosed vessels (left gastric, splenic, common hepatic, and superior mesenteric), which independently originate from the abdominal aorta. Some common variations, such as hepatomesenteric trunk or independent origin of left gastric artery, are well accounted for using this classic model, although it does not explain the formation of some rare variations such as hepatogastric or splenomesenteric trunk formation. Previously, we presented typological diagrams of the phrenico-celiaco-mesenteric arterial system [8, 9, 15]. These

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## Obesity and the Risk of Diabetes Mellitus in Middle-aged Japanese Men

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The morbidity of diabetes mellitus is increasing gradually in Japanese populations. It is important to clarify the risk factors of diabetes in Japanese populations in order to take adequate measures against the increasing morbidity of diabetes. In order to evaluate the link between past and concurrent obesity and diabetes in middle-aged Japanese men, we conducted a worksite-based historical cohort study in Okayama, Japan in 1999. Annual health examination data of middle-aged male workers in a worksite were collected. The relative risks of past and concurrent obesity for developing diabetes were calculated. Subjects with a past history of obesity at between 40 and 50 years of age had a significantly higher risk of developing diabetes by age 55 than did subjects in the normal weight group. These results suggest that, in order to prevent diabetes in middle-aged Japanese men, health guidance for normal weight maintenance should be provided not only for middle-aged men, but also for men under age 40.

**Key words:** obesity, body mass index, diabetes mellitus, cohort study

**D**iabetes mellitus is a common disease. As of 1999, there were 2.1 million diabetic patients in Japan [1]. A study conducted by the Japanese Ministry of Health and Welfare estimated that there were 6.9 million people diagnosed with, or highly suspected of having, diabetes in Japan [2]. The number of patients has been increasing gradually, and it has been estimated that the number of patients diagnosed with or highly suspected of having diabetes will reach more than 10 million within 10 years [3].

The most common form of diabetes mellitus in the Japanese population is type 2 diabetes. It is well known

that this type of diabetes has several risk factors, such as a family history of the disease, male sex, advancing age, and some environmental- and lifestyle-related factors [2, 4-10]. The main reasons for the increasing incidence of diabetes in the Japanese population are considered to be environmental and lifestyle changes [3].

Among the lifestyle-related factors, obesity is reported to be one of the strongest risk factors for developing type 2 diabetes [10-15]. It is also reported that type 2 diabetes is more strongly associated with past than with concurrent obesity [14-16]. However, most of these reports are from North America or Europe. And while it is reported that different ethnic groups share similar risk factors for diabetes, the impact of these factors differs between ethnic groups [4, 5]. To reduce the increasing prevalence of diabetes in Japan, it is therefore important

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## Daily Low-Dose Cisplatin and Concurrent Thoracic Irradiation for Poor-Risk Patients with Unresectable Non-Small-Cell Lung Cancer

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A pilot study was conducted to assess the efficacy and feasibility of daily low-dose cisplatin with concurrent thoracic irradiation for clinically unresectable non-small-cell lung cancer (NSCLC). Patients with inoperable NSCLC who had poor risk factors such as advanced age, poor performance status, poor lung function, or concomitant active malignancy were entered into the study. Low-dose cisplatin (6 mg/m<sup>2</sup>) was administered daily before concurrent thoracic irradiation (2 Gy/day; total dose of 60 Gy) was given. Twenty-five patients were registered. The majority of the patients had either stage IIIA (24.0%) or stage IIIB (60.0%) disease. Fifteen patients (60.0%) completed the planned treatment. Both chemotherapy and radiotherapy were stopped in 3 patients (12.0%) due to poor response, and 7 patients (28.0%) partly received radiotherapy alone as a result of their toxicity response. The proportion of total administered dose to planned dose was 90.9% for chemotherapy and 99.3% for radiotherapy, which were comparable to those in previous studies for LA-NSCLC patients without poor risk factors. Grade 3 leukopenia and neutropenia developed in 14 patients (56.0%) and 10 patients (40.0%), respectively, but grade 4 toxicity was not encountered. Grade 3 pneumonitis and esophagitis were observed in 4 patients (16.0%) and 2 patients (8.0%), respectively. The overall response rate was 60.0%. The median survival time was 22 months, and the 2-year survival rate was 50.3%. Daily low-dose cisplatin and concurrent thoracic irradiation were well tolerated even by poor-risk patients with NSCLC, and showed a therapeutic efficacy similar to that for good-risk patients.

**Key words:** non-small-cell lung cancer, concurrent chemoradiotherapy, low-dose cisplatin, poor-risk factor

**L**ung cancer is the leading cause of cancer-related death in industrialized countries [1]. Surgery

offers the best chance for the cure of early-stage non-small-cell lung cancer (NSCLC). However, only a minority of patients are diagnosed with operable disease, and approximately one-third of patients have unresectable locally advanced disease at the time of diagnosis. Although these patients have been previously treated with

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Case Report

## Anterior Intercostobrachial Nerve Penetrating the Pectoralis Minor or Major Muscle

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Two previously unknown anomalies of the anterior intercostobrachial nerve were described. In one case, the anterior intercostobrachial nerve penetrated the pectoralis minor muscle. In the other case, it penetrated the pectoralis major muscle. In both cases, the anomalous nerve supplied the skin of the upper arm.

**Key words:** anomalies of intercostobrachial nerve, lateral cutaneous branch of the second intercostal nerve, pectoralis minor muscle, pectoralis major muscle

Lateral cutaneous branches of the intercostal nerves issue the anterior and posterior rami [1]. The anterior and posterior rami of the 2nd intercostal nerve ran in the axilla as the anterior and posterior intercostobrachial nerves, and supply the skin of the axilla and the arm [1, 2]. The posterior intercostobrachial nerve anastomoses into the medial cutaneous nerve of the arm, while the anterior one is independent [1-3]. This paper describes two cases of previously unknown anomalies in which the anterior intercostobrachial nerve penetrates the pectoralis minor or major muscle.

### Case Report

The anomalies were found in a 79-year-old Japanese male who died in 1998 of gastric cancer (Case A), and in a 83-year-old Japanese female who died in 2000 of heart failure (Case B). These subjects had no history of surgical procedures. They were fixed conventionally by arterial perfusion with 10% formalin, and subsequently dehydrated with 50% ethanol. Dissections were performed

in the 1998 (Case A) and 2000 (Case B) clinical anatomy courses for medical students at Okayama University Medical School.

No marked anomalies were found in the plexus brachialis on either side of these 2 cases. Neither marked anomalies of the subclavian and axillary arteries nor marked anomalies of the pectoralis minor and major muscles were found on either side of either case.

**Case A.** The anterior intercostobrachial nerve (anterior ramus of the lateral branch of the right 2nd intercostal nerve) ran along the posterior surface of the right pectoralis minor muscle, and penetrated the lower segment of this muscle from the dorsal aspect (Figs. 1A, 1B). It independently (without any anastomosis with other nerves) supplied the skin of upper segments of the right arm from the medial aspect (Fig. 1). It issued no muscular branches.

The posterior branch of the lateral cutaneous branch of the 2nd intercostal nerve (posterior intercostobrachial nerve) was well developed, and anastomosed into the medial cutaneous nerve of the arm.

**Case B.** The anterior intercostobrachial nerve (anterior ramus of the lateral branch of the left 2nd

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