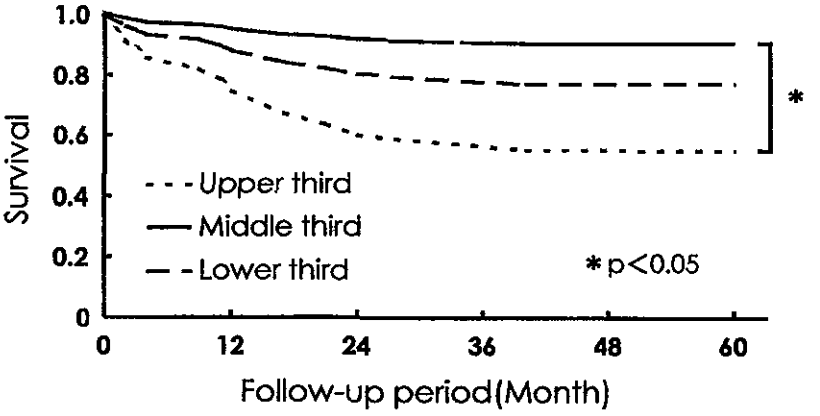

Fig. 2: Age- and sex-adjusted survival curves according to the location of gastric cancer.

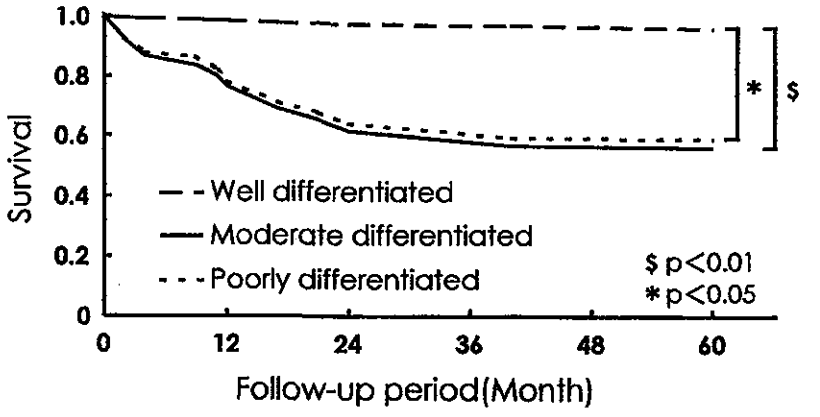

Fig. 3: Age- and sex-adjusted survival curves according to histological type of gastric cancer.

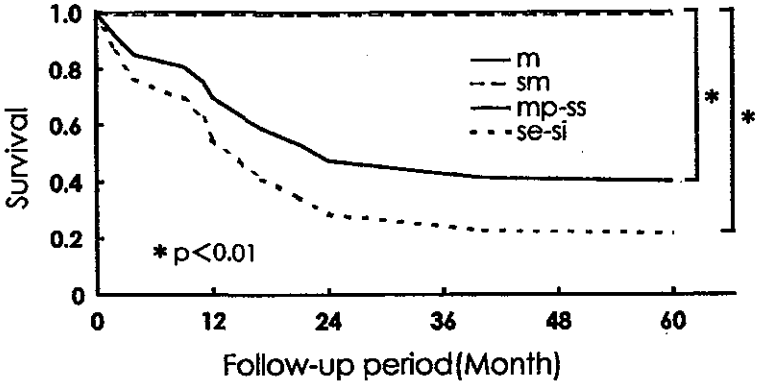

Fig. 4: Age- and sex-adjusted survival curves according to the depth of invasion of gastric cancer. m; confined to the mucosa, sm; limited to the submucosa, mp-ss; limited to the proper muscular or subserosal layer, se-si; beyond the serosa.

厚生労働科学研究費補助金（健康科学総合研究事業）
アンジオテンシン変換酵素遺伝子多型と脳・心血管病の関係に関する疫学調査：
久山町研究

分担研究報告

久山町剖検例におけるアンジオテンシン変換酵素遺伝子多型の測定

分担研究者　中別府　雄作　九州大学・生体防御医学研究所・教授

研究要旨　過去40年間に剖検した久山町住民のパラフィン包埋組織からのDNAの抽出法および遺伝情報の増幅法を確立した。さらに,1961年から32年間の追跡調査間の久山町剖検例968例のパラフィン標本からアンジオテンシン変換酵素（ACE）遺伝子I/D多型を決定し,癌死とACE遺伝子多型の関連を解明するためのデータベースを構築した。

A．研究目的

過去40年間に剖検した久山町住民のパラフィン包埋組織からのDNAの抽出法および遺伝情報の増幅法を確立したので，この手法を用いて剖検組織から遺伝子解析を行い，その結果と生活習慣病との関連を検討する目的で臨床データとともにデータベースの構築を目指した。

B．研究方法

ホルマリン固定パラフィン包埋ブロック組織からのDNA抽出は，SDS-Percoll-Chloroform-GuSCNを用い，KURABOの自動DNA抽出器を用いて行った。遺伝子型はEvansら（1994）の報告した方法に従いNested-PCR法を使用して判定した。判定された遺伝子型の正確さを検証するために、新鮮凍結組織の解析からACEの多型が決定されていた18例についてホルマリン固定パラフィン包埋ブロック組織を用いて検討した。また，パラフィン包埋ブロック組織から抽出したDNAからnested PCRにより増幅されたDNA断片の配列を直接制限酵素による切断部位の存在を比較する事で確認した。

（倫理面への配慮）本研究は3省合同の「倫理指針」に準拠し，九州大学医学部倫理委員会の承認の元で行われた。

C．研究結果

1961年から32年間の追跡調査間の久山町剖検例968例のパラフィン標本において,ACE遺伝子I/D多型を解析した。その結果、98.5%の標本で遺伝子型を決定することができた。940例のパラフィン包埋ブロック組織を用いて判定したACE遺伝子多型の頻度は,DD型12.4%,ID型47.3%,II型40.3%であった。この分布は,Hardy-Weinbergの平衡と矛盾するものではなかった（$\chi^2_{2df} = 0.67$, $p = 0.72$）。

32年間の追跡調査の期間に117名が癌死と診断されていることから,癌死とACE

遺伝子多型の関連を明らかにするために性別，年齢，body mass index，耐糖能異常，血清総コレステロール，収縮期血圧，アルコール摂取，喫煙の生活習慣および関連の臨床データとともに，ACE 遺伝子 I/D 多型のデータベースを構築した。

D．考　察

癌死と ACE 遺伝子多型の関連を性別，年齢，アルコール摂取，body mass index，耐糖能異常，血清総コレステロール，収縮期血圧，喫煙の生活習慣および関連の臨床データで補正しながら解析中であるが，DD の遺伝子型が II および ID の遺伝子型に比べて喫煙者の癌死の危険因子となる可能性が示唆されており，来年度にかけて最終的な解析結果をまとめる予定で，解析を進めている。

E．結　論

過去 40 年間に剖検した久山町住民のパラフィン包埋組織からの DNA の抽出法および遺伝情報の増幅法を確立した。1961 年から 32 年間の追跡調査間の久山町剖検例 968 例のパラフィン標本から ACE 遺伝子 I/D 多型を決定し，癌死と ACE 遺伝子多型の関連を解明するためのデータベースを構築した。

F．健康危険情報

なし。

G．研究発表

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2．学会発表

特になし

H．知的所有権の取得状況

1．特許取得　　なし

2．実用新案登録　なし

厚生労働科学研究費補助金（健康科学研究事業）
分担研究報告書

「機能的」脈管リモデリングにおける血管・リンパ管内皮細胞の機能制御機構

分担研究者　居石克夫　九州大学大学院医学研究院・教授

研究要旨　本研究では、動脈硬化、血栓症などの血管リモデリングに伴う機能的血管・リンパ管新生過程における、各種脈管新生因子の発現動態の解析、ならびにその制御機構における血小板由来増殖因子（PDGF-A, -B）を中心にした病態学的意義について検討した。血管内皮増殖因子群（VEGF、HGF）は間葉系細胞のPDGF-Aを介したオートクライン制御により血管新生を促進すること、また内皮由来PDGF-Bが間葉系細胞のVEGF-Cを誘導、さらにこのVEGF-Cは内皮由来のPDGF-Bを誘導し、このパラクライン制御系が毛細血管の成熟性促進とリンパ管形成に密に関与していることが明らかになった。

A．研究目的

我々はこれまでFGF-2が広い安全域と虚血肢に対する高い救肢効果を示しこと、また血流回復を伴う「機能的血管新生」の重要性を提唱して来た。このFGF-2の機能として内因性血管新生因子群の階層的発現制御を誘導することを明らかにして来た。本研究では、この「階層的血管新生発現制御機構」における血小板由来増殖因子（PDGF）の機能について、これ迄の研究をさらに発展させた。

B．研究方法

雄性C57BL/6マウスに重症虚血モデルを作成、SeV-FGF2あるいはSeV-luciferaseを筋注した。血流回復効果はレーザードップラー法、各種血管新生因子の発現の経時的変化をreal-time PCR法、ELISA法にて定量化した。各種リガンド、受容体に対する中和活性を持つ特異抗体にて活性を遮断し、遺伝子発現量、治療効果をモニターした。

（倫理面への配慮）

本研究は九州大学組み換えDNA実験委員会の承認にもと、P2動物実験室で施行した。動物実験は、九州大学動物実験委員会の審議・許可を得た。

C．研究結果

1．PDGF-Aについて：

In vitro、in vivoにおいて、FGF-2はVEGF、HGF、PDGF-Aの発現を誘導し、PDGF-A、p70S6Kの活性遮断によりVEGF、HGFの発現増強効果は消失した。このPDGF-A/p70S6K系は悪性腫瘍においてもVEGF発現を支配する、恒常的な系であることが明らかとなった。

2．PDGF-Bについて：

FGF-2の遺伝子導入により、内因性の

VEGF-C、PDGF-B の発現が増強した。VEGF-C の受容体 VEGFR3/FLT-4 活性中和抗体投与により、FGF-2 の治療効果が消失、PEGF-B の発現も低下し、多数の微小血管瘤が形成されると共に、リンパ管の数が減少した。一方、PDGF-B の発現を特異的活性中和抗体で遮断しても、VEGF-C の発現は低下した。

D．考察、E．結論

FGF-2 により、階層的・多段階的に内因性血管新生因子群が誘導され、その制御に PDGF が重要であることが明らかとなった。このシステムの破綻が、血管数と血流の乖離に至ることも示された。

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2．学会発表

日本病理学会、日本動脈硬化学会など約 50 件。

H．知的財産権の出願・登録状況（予定を含む。）　特になし。

厚生労働科学研究費補助金・健康科学研究事業
アンジオテンシン変換酵素遺伝子多型と脳・心血管病の関係に関する疫学調査：
久山町研究

分担研究報告書

ヒト悪性腫瘍の細胞学的特性および悪性度と遺伝子異常に関係する検討

分担研究者：恒吉正澄（九州大学大学院医学研究院形態機能病理学・教授）

研究要旨
消化管と骨軟部組織に発生するさまざまな腫瘍において種々の細胞増殖関連因子や遺伝子異常について調べ、それらの因子と腫瘍の発生から発育進展様式や臨床病理学的事項との関連を解析した。
さまざまな悪性腫瘍において遺伝子異常と腫瘍の発育進展との関連が見いだされ、とくに腫瘍の種類により特異的な遺伝子異常が生じていることが示唆された。また、細胞分化（発現形質）や細胞増殖動態と遺伝子異常の関連の解析も腫瘍の生物学的態度を知る上で重要であると考えられた。

A. 研究目的

消化管と骨軟部組織に発生するさまざまな腫瘍において種々の細胞増殖関連因子や遺伝子異常を調べ、それらの因子と腫瘍の発生から発育進展様式および臨床病理学的事項との関連について解析し、腫瘍の悪性度評価に応用することを目的とする。

B. 研究方法

消化管の腫瘍は、胃癌を用い、軟部腫瘍は平滑筋肉腫や滑膜肉腫、横紋筋肉腫、悪性末梢神経鞘腫瘍などを用いた。消化管腫瘍では、gastric mucin（胃腺窩上皮のマーカー）、MUC2（腸杯細胞のマーカー）、CD10（小腸刷子縁のマーカー）による免疫染色を行い、胃型・腸型の形質発現と腫瘍の発育進展様式や悪性度、細胞増殖との関連や、細胞増殖・アポトーシスや炎症、血管新生と関連する因子である NF-κBの意義を解析した。

軟部腫瘍では、遺伝子異常（p53、MDM2、p14、p16、マイクロサテライト不安定性）や細胞増殖関連因子（MIB-1、E2F-1）の腫瘍の発育における意義や悪性度との関連を解析した。

なお、マイクロサテライト不安定性の解析には、hMLH1 と hMSH2 に対する抗体を用いた免疫染色とマイクロサテライトマーカーを用いた PCR 法により行った。p14、p16、p53 の解析はそれぞれに対する抗体を用いた免疫染色に加え、腫瘍からの DNA 抽出を行いメチレーションと遺伝子変異をオートシークエンサーにより検出した。

また、消化管に発生する Gastrointestinal stromal tumor (GIST)と胃腸管外に発生する GIST の遺伝子異常と細胞増殖動態と悪性度に関する解析も行った。

C. 研究結果

1. 胃癌の発育進展における形質変化と細胞増殖。

粘膜下浸潤胃癌の分化型腺癌の解析で、粘膜内から粘膜下層へ浸潤しさらに

リンパ節へ転移する場合、分化型の形態を保持しながら形質発現は減弱または消失するものがあることが判明した。さらに、隆起型と陥凹型の胃癌では細胞増殖活性とアポトーシスの程度が異なり、陥凹型胃癌のほうが悪性度が高いことが示唆された。

2. 胃癌におけるNF-κBの意義。

胃癌においてNF-κBは予後因子の一つであることが多変量解析により示され、さらにNF-κBの活性化にはIL-1βが重要な役割を果たしていることも判明した。

3. 軟部腫瘍における細胞増殖関連因子、種々の遺伝子異常、マイクロサテライト不安定性の解析。

軟部平滑筋肉腫ではp53遺伝子異常を示すものは予後不良であり、頻度は少ないがdeath-associated protein (DAP) kinase遺伝子異常を示すものも予後不良であった。

滑膜肉腫ではPTEN遺伝子およびp53、APC、E-cadherinなどの癌抑制遺伝子の何らかの異常を約半数に認めたが予後との相関はなかった。また、E-cadherin機能喪失は遺伝子変異もしくはS-nail発現によるE-cadherin遺伝子抑制による機序が示唆された。

明細胞肉腫では、p53遺伝子あるいはp16/p14遺伝子異常は核分裂や腫瘍壊死と相関し、予後に悪影響を与える因子と考えられた。

横紋筋肉腫では、p53およびMDM2蛋白過剰発現の間に相関を認め、それらの過剰発現群ではMIB-1標識率は高値を示した。E2F-1標識率は胎児型より胞巣型で高値であった。

種々の軟部肉腫においてマイクロサテライト不安定性は、hMSH2もしくはhMLH1蛋白発現減弱と相関し、DNAミスマッチ修復遺伝子の不活性化がマイクロサテライト不安定性の原因と考えられた。また、YB-1の核内移行は抗癌剤の多剤耐性に関与するp糖蛋白発現と相関した。

4. 消化管GISTと消化管外GISTの遺伝子異常および細胞増殖動態と悪性度の関連。

消化管外GISTにおいても消化管GIST同様にc-kitあるいはPDGFRAの遺伝子変異を認めることが判明し、消化管GISTでは従来の悪性度判定因子に加え細胞増殖に関連するcyclin A、cycliln B1とcdc2が有用であることを報告した。

D. 考察

胃癌の解析では、発育進展の過程で、形態は保持したまま形質発現の減弱・消失を認めるものは、高悪性度の指標となると考えられた。また、隆起型と陥凹型の胃癌では形質発現には違いが見られなかったが、細胞増殖活性とアポトーシスの違いが発育形態と悪性度を規定していることが想定された。胃癌の形質発現や細胞増殖動態の解析がその生物学的態度を調べる上で重要であると考えられた。

軟部腫瘍の解析では、腫瘍の発生から発育進展においてマイクロサテライト不安定性や種々の遺伝子異常が関与し、それらと高悪性度との関連がわかってきた。そして、それらを規定するさまざまな因子の相互関係も少しずつ解明されてきた。今後は、各腫瘍に特異的な異常と共通する異常をもっと明らかにして行く必要がある。

GISTは消化管のみでなく腹腔内にも発生し消化管GISTと同様の遺伝子変異を有することとGISTの悪性度評価にcyclin Aも有用であることが判明し、この腫瘍の治療戦略に有益な研究となったと言える。

E. 結論

さまざまな悪性腫瘍において遺伝子異常と腫瘍の発育進展との関連が見いだされ、とくに腫瘍の種類により特異的な遺伝子異常が生じていることが示唆された。また、細

胞分化(発現形質)や細胞増殖動態と遺伝子異常の関連の解析も腫瘍の生物学的態度を知る上で重要であると考えられた。

F. 研究発表

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ORIGINAL ARTICLE

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healthsciences

Incidence and Prognosis of Gastric Cancer in a Population-based Cohort Survey: The Hisayama Study

K. Tanaka, Y. Kiyohara, I. Kato, T. Matsumoto, H. Yamagata, M. Kubo, Y. Tanizaki, K. Okubo, H. Nakamura, H. Iwamoto, K. Nakayama & M. Iida
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Tanaka K, Kiyohara Y, Kato I, Matsumoto T, Yamagata H, Kubo M, Tanizaki Y, Okubo K, Nakamura H, Iwamoto H, Nakayama K, Iida M. Incidence and prognosis of gastric cancer in a population-based cohort survey: The Hisayama study. Scand J Gastroenterol 2004;39:459–463.

Background: No population-based cohort studies have been undertaken to evaluate the incidence and prognosis of gastric cancer. The purpose of this investigation was to clarify the incidence and fatal prognosis of gastric cancer and to determine the factors that contribute to the prognosis in a general Japanese population in Hisayama using a prospective study design. **Methods:** From 1988 to 1998 a total of 2605 subjects aged 40 years or older with no history of gastrectomy or gastric cancer were followed-up prospectively after a health examination. The diagnosis of gastric cancer was based on clinical records or autopsy findings. **Results:** During the follow-up period, 76 subjects developed gastric cancer. The age-adjusted incidence of gastric cancer for men (4.9 per 1000 person-years) was 4-fold higher than that for women (1.2, $P < 0.05$). In men, the incidence of gastric cancer increased with advancing age, but this trend was not observed in women. The age- and sex-adjusted 5-year survival rate was significantly higher in cancers of the middle third of the stomach than in those of the upper third of the stomach. The survival rate was higher in cancers of well-differentiated adenocarcinoma than in those of the other histological types. There were no cases of cancer-related death among the early gastric cancers during the follow-up period. **Conclusions:** Our data suggest that men are at higher risk of gastric cancer than women in the general Japanese population. Clinical stage, histological type, and site of cancer in the stomach contribute to a fatal prognosis.

Key words: Cohort study; gastric cancer; incidence; mortality

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In Japan, gastric cancer is one of the most common malignant neoplasms in both men and women (1). The age-adjusted mortality from gastric cancer among Japanese has been shown to have declined conspicuously during the past 25 years (2–4), though it is still the highest in the world (4). The establishment of a mass screening program for gastric cancer and advances in therapy have been suggested to contribute to this decrease in the mortality rate (5, 6).

There have been several registration studies regarding the incidence (2–4, 7–9) and prognosis (5, 6) of gastric cancer in Japan. However, study designs such as those may have some limitations; these studies did not include concealed cancers, because autopsy examinations are not commonly performed (10), and the registration rate is not high, being approximately 50% (11). Therefore, it is possible that the incidence is underestimated in these studies. We thus performed a prospective cohort study of a defined Japanese population in which we sought to examine the development of gastric cancer and to verify the causes of death in 77% of the subjects by autopsy. We undertook this study in order to determine the true incidence and prognosis of gastric cancer as well as its prognostic factors in the general population.

Subjects and Methods

Study population

Hisayama is a subrural town on Kyushu Island in the southern part of Japan, and is adjacent to Fukuoka City, a large urban center. The population of the town is approximately 7500 and has been stable for the past 30 years. According to the 1985 census, the ages and occupational distributions of the population are almost identical to those of Japan as a whole (12). The dietary pattern of the residents was also found to be equivalent to that of the participants of the National Nutrition Survey, which selected subjects from 300 areas throughout Japan (13).

The present study was based on a prospective population survey that has been conducted in Hisayama since 1961 (14). In 1988, 2742 Hisayama residents aged 40 years or older

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(80.1% of the total population in that age group) underwent a health check-up which did not include an X-ray or endoscopic examination of the stomach. After excluding 132 individuals who had a history of gastrectomy and/or gastric cancer and 5 who died during the examination period, a total of 2605 subjects (1071 M, mean age 57 years; 1534 F, mean age 59 years) were enrolled in the study.

Follow-up survey

The study population was followed up for 10 years from 1 December 1988 until 30 November 1998. The subjects underwent repeated health check-ups that were performed every year. Approximately 60% of the subjects regularly returned for the check-ups, while 149 subjects (5.7% of the total subjects) moved out of Hisayama during the follow-up period. The health status of the subjects who did not undergo regular check-ups or who moved out of town was checked every year by mail or telephone. In addition, a daily monitoring system was established by the study team and local physicians or members of the Division of Health and Welfare of Hisayama Town. We are unable to follow-up on only one of the subjects in the cohort. To identify new occurrences of gastric cancer in the cohort, we monitored the records of barium meal examinations, upper endoscopy examinations and, when necessary, biopsy diagnoses at local clinics or general hospitals in and around Hisayama. We also checked all of the records of annual mass screenings for gastric cancer using barium examination. Furthermore, in order to find any concealed gastric cancer, autopsies were performed on 304 (77.4%) of a total of 393 subjects who died during the follow-up period. The diagnosis of all cases of gastric cancer was confirmed by histological examination of specimens obtained by gastrectomy, endoscopic mucosal resection, or autopsy. Pathological diagnosis and classification of the identified gastric cancers were made according to the guidelines proposed by the Japanese Research Society for Gastric Cancer (15) and the histological classification of Laurén (16). All gastric cancers identified were of the common type as defined by the Japanese guidelines, and included papillary adenocarcinoma, well-differentiated adenocarcinoma, moderately differentiated adenocarcinoma, poorly differentiated adenocarcinoma, signet-ring cell carcinoma, and mucinous adenocarcinoma. According to the histological type identified by Laurén, the cancers were classified into differentiated type and undifferentiated type (poorly differentiated) adenocarcinoma. The differentiated type was further divided into well- and moderately differentiated adenocarcinoma based on the Japanese guidelines (6, 15). The tumor location was determined through a combined evaluation of the clinical and pathological records.

Statistical analysis

The incidence of gastric cancer was calculated using the person-year method, and differences in incidence between subgroups of the population were analyzed with the Cox proportional hazards model (17). The age- and sex-adjusted survival rates by tumor location and histological type were also calculated and compared using the Cox proportional method. The survival rates by depth of invasion were compared using the log-rank test, since none of the patients with early gastric cancer died during the follow-up period and thus the Cox proportional method could not be applied. When estimating survival rates in subjects with two synchronous gastric cancers, we considered the more advanced cancer as the index tumor. The world standard population was used for age adjustment. The significance was indicated by a P value of less than 0.05.

Results

During the follow-up period, gastric cancer was diagnosed in 76 subjects (54 M, 22 F) and among them 7 patients (9.2%) had two synchronous gastric cancers. Thus, a total of 83 cases of gastric cancer were found. The mean age at the diagnosis of cancer was 66.2 years for men and 69.1 years for women. There were three cases (3.9%) of concealed cancer that were not found until autopsy. The time interval from the baseline to the diagnosis of gastric cancer ranged from 0.5 to 9.8 years (mean, 5.8 years).

The clinicopathological findings of gastric cancer by gender are presented in Table I. The location and histological type of cancer did not differ between the sexes. A total of 56 (67.5%) cancers were confined to the submucosal layer, and were thus regarded as early gastric cancer. The frequency of occurrence was the same between men and women (66.7% versus 69.6%). The proportion of patients treated surgically was 88.7% in men and 85.0% in women.

The age-adjusted incidence of gastric cancer was 4.9 and 1.2 per 1000 person-years for men and women, respectively;

Table I. Clinicopathological characteristics of gastric cancer, 1988 to 1998

	Men ($n = 60$) n (%)	Women ($n = 23$) n (%)
Location		
Upper third	11 (18.3)	3 (13.0)
Middle third	24 (40.0)	6 (26.1)
Lower third	25 (41.7)	14 (60.9)
Histological type		
Well-differentiated	32 (53.3)	14 (60.9)
Moderately differentiated	13 (21.7)	4 (17.4)
Poorly differentiated	15 (25.0)	5 (21.7)
Depth of invasion		
Limited to the mucosa	29 (48.3)	12 (52.2)
Limited to the submucosa	11 (18.3)	4 (17.4)
Limited to the subserosa	11 (18.3)	4 (17.4)
Beyond the serosa	9 (15.0)	3 (13.0)
Therapy (without unsuspected cancer)		
Operation	36 (67.9)	15 (75.0)
Operation after EMR*	2 (3.8)	0 (0)
EMR	9 (17.0)	2 (10.0)
Observation	6 (11.3)	3 (15.0)

* EMR = endoscopic mucosal resection.

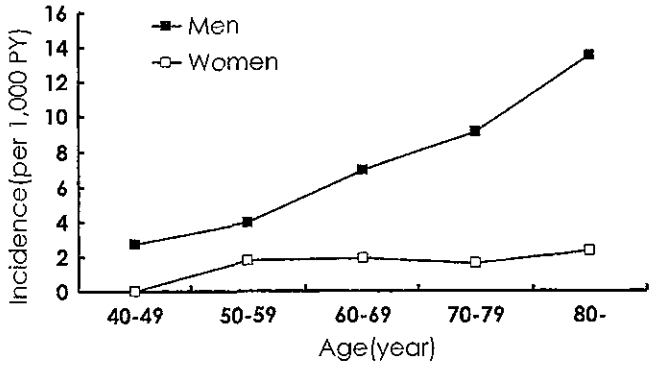

Fig. 1. Age-specific incidence of gastric cancer in men (filled squares) and in women (open squares) during a 10-year follow-up: the Hisayama study.

the incidence was significantly higher in men than in women ($P < 0.05$). The age-specific incidences of gastric cancer by gender are shown in Fig. 1. The incidence increased with advancing age in men, but this trend was not observed in women.

When gastric cancer-related deaths were considered as an end-point, the 5-year survival rate was 75.9% for subjects with gastric cancer. The rate did not differ between men and women (76.0% versus 75.5%). The age- and sex-adjusted survival curves according to the location of the cancer in the stomach are shown in Fig. 2. The 5-year survival rate was 55.4% for cancers in the upper third of the stomach, 90.6% for those in the middle third, and 77.3% for those in the lower third. The survival rate in cancers of the upper third was significantly lower than that in cancers of the middle third hazard ratio (HR), 6.0; 95% confidence interval (CI), 1.1–32.9; $P < 0.05$. However, the survival rate in cancers of the lower third did not differ significantly from that in cancers of the middle third (HR, 2.6; 95% CI, 0.5–13.0; $P = 0.24$). The frequency of early gastric cancer was 50.0% for the upper third of the stomach, 84.6% for the middle third, and 54.3% for the lower third, while the frequency of well-differentiated adenocarcinoma was 41.7%, 50.0%, and 57.1% for the upper, middle, and lower thirds, respectively.

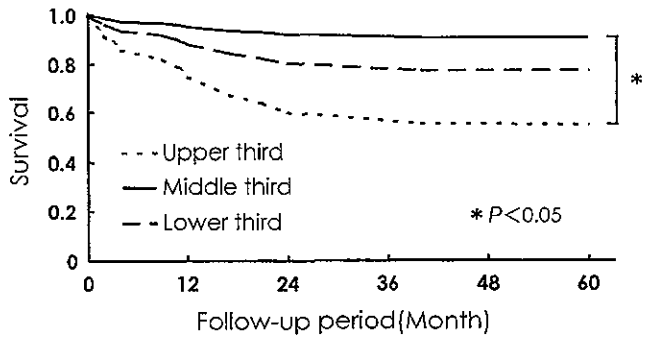

Fig. 2. Age- and sex-adjusted survival curves according to the location of gastric cancer: the Hisayama study.

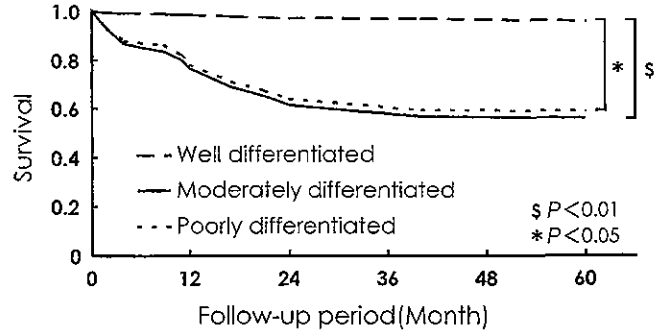

Fig. 3. Age- and sex-adjusted survival curves according to histological type of gastric cancer: the Hisayama study.

The age- and sex-adjusted survival curves according to the Japanese histological classification are shown in Fig. 3. The 5-year survival rate was 97.1% for well-differentiated adenocarcinoma, 58.1% for moderately differentiated adenocarcinoma, and 60.1% for poorly differentiated adenocarcinoma. The survival rates for moderately and poorly differentiated adenocarcinomas were significantly lower than those for well-differentiated adenocarcinoma (HR, 18.5; 95% CI, 2.2–157.6; $P < 0.01$ for moderately differentiated adenocarcinoma and HR, 16.9; 95% CI, 2.0–146.0; $P < 0.05$ for poorly differentiated adenocarcinoma). However, the survival rates did not differ significantly between the last two groups. According to the histological type described by Laurén, the 5-year survival rate was 86.9% for differentiated-type adenocarcinoma and 60.1% for undifferentiated-type adenocarcinoma. The survival rates for undifferentiated-type adenocarcinoma were significantly lower than those for differentiated-type adenocarcinoma (HR, 3.5; 95% CI, 1.1–11.6; $P < 0.05$).

The age- and sex-adjusted survival curves according to the invasion depth are shown in Fig. 4. The 5-year survival rate was 100% for early gastric cancer limited to the mucosa or submucosa, 41.2% for advanced gastric cancer confined to the subserosal layer, and 22.7% for more advanced cancer. The survival rate for early gastric cancer was significantly higher than that for the other groups ($P < 0.01$).

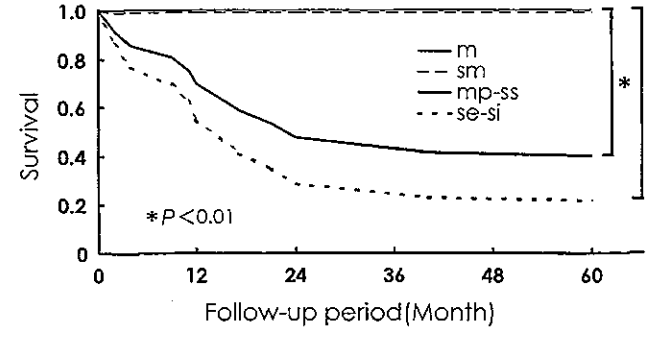

Fig. 4. Age- and sex-adjusted survival curves according to the depth of invasion of gastric cancer: the Hisayama study. M = confined to the mucosa; sm = limited to the submucosa; mp-ss = limited to the proper muscular or subserosal layer, se-si = beyond the serosa.

Discussion

Our data indicate that the incidence of gastric cancer was 4-fold higher in men than in women; however, the clinicopathological characteristics of the cancer did not differ between the sexes in a general Japanese population. It is also noteworthy that the prognosis of gastric cancer was more favorable in our subjects than in previous reports (5, 6).

It has been reported previously that the incidence of gastric cancer in the Japanese population aged 40 years or older is 3.7–4.9 per 1000 person-years for men and 1.6–2.0 for women (1). The incidence of gastric cancer of 4.9 in our men was equivalent to that reported previously, but the value of 1.2 in our women was much lower. It is also characteristic that the incidence of gastric cancer in our women did not increase with advancing age. These findings suggest that the pathogenesis of gastric cancer is different between the sexes. In an experimental induction of cancer in the rat stomach, Furukawa et al. (18) have shown that sex hormones are crucial determinants of the sex difference in the risk of gastric cancer. In our subjects, positive IgG antibodies to *Helicobacter pylori* are more common in men than in women (71.5% versus 62.4%, $P < 0.01$) (19); however, the prevalence of *H. pylori* infection alone cannot explain the sex difference in the risk of gastric cancer. This finding confirms that *H. pylori* infection is not the only culprit in the etiopathogenesis of the cancer. On the other hand, we have previously shown that in our study population, the frequencies of potential risk factors for gastric cancer, namely, smoking habits and alcohol consumption, differ between men and women. It thus seems likely that the sex difference in the incidence of gastric cancer is due to differences in lifestyle known to be associated with gastric cancer (19–21). Further analyses of these factors are needed to provide a better understanding of the pathogenesis of this disease.

The incidence of gastric cancer in Japan is the highest among Western and Asian countries (1). It can be assumed that environmental and genetic factors contribute significantly to the high incidence of this disease. A registration study of Japanese emigrants to the United States revealed that the incidence of gastric cancer in the second generation of emigrants was approximately half of that in the first generation (20). These results suggest that environmental factors play an important role in the development of gastric cancer. In addition to the high prevalence of *H. pylori* infection (19, 22–24), other factors such as a high intake of salt (21, 25) and starch (25, 26), a lower intake of vegetables and fruits (21, 27, 28), and a higher frequency of smoking (26–28) are considered to contribute to the increased risk of gastric cancer in the Japanese.

In our cohort, the 76% 5-year survival rate for gastric cancer was more favorable than that observed in previous studies. Msika et al. (29) have reported in a registration study of local residents in France that the 5-year survival rate is approximately 30% among subjects with gastric cancer, 50% of whom have been treated curatively. In a defined Swedish population, the frequency of early gastric cancer among all gastric cancers was only 16% (30), and the 5-year survival rate was approximately 20% for advanced gastric cancers and 60% for early cancers. In our subjects, early gastric cancers accounted for 71% of all gastric cancers, and it is therefore likely that the better survival among our subjects was due in part to the predominance of early cancers. Another factor contributing to the better prognosis for the gastric cancer cases in our study is that no patients with early gastric cancer died during the follow-up period, suggesting that the treatment was adequate.

In our subjects, as has been reported previously (6, 29, 31), the prognosis for cancers in the middle third of the stomach is more favorable than that for cancers occurring at other sites. This finding can be explained partly by the invasion depth of the tumor, as early cancers were found to be the most common in the middle third of the stomach. These results suggest that cancer in the middle third of the stomach is detected more readily than that at other sites. Alternatively, deeper invasion and a lower frequency of well-differentiated histology seem to be factors in the poor prognosis for cancer in the upper third of the stomach. Atrophic gastritis is related primarily to the differentiated type of gastric cancer (32), and gastritis is rare in the upper third of the stomach, resulting in a less frequent well-differentiated histology at this site.

We classified the differentiated type of gastric cancer, based on Laurén's classification (16), into two categories: well-differentiated or moderately differentiated adenocarcinoma. These subclassifications provide evidence that the prognosis for the moderately differentiated histology is poorer than that for the well-differentiated histology but that it is similar to the prognosis for the poorly differentiated histology. These findings are in accordance with those of a previous study of 10,000 consecutive patients with gastric cancer who underwent primary gastrectomy (6). It thus appears that Laurén's classification (16) may not be appropriate as a prognostic factor from an epidemiological perspective.

Our study has several limitations. First, our survey area may not be representative of the entire Japanese population. However, the incidence of gastric cancer in Fukuoka prefecture, in which Hisayama town is located, is not obviously different from that observed in a nationwide survey (33). Second, we may have overestimated the incidence of gastric cancer, as we could not exclude individuals who had concealed cancer at baseline. However, overestimations of this kind are a common problem in any type of epidemiological study. Third, there was a small number of cases of gastric cancer in our cohort, indicating a high possibility of bias in our results for the incidence and prognosis. Nonetheless, we believe that the findings of our study represent an accurate incidence and prognosis, since we performed the study using a highly accurate method for determining all gastric cancer cases.

In conclusion, the findings of our population-based cohort study suggest that men are at a higher risk for gastric cancer than women, and that the risk of gastric cancer in women does not increase with advancing age in the general Japanese population. In addition, the prognosis of gastric cancer differs according to clinicopathological variables such as invasion depth, location, and histological grade. As such, the differentiated type of cancer as defined by Laurén's classification needs to be revised, with further consideration given to the prognosis of gastric cancer.

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Comparison of diagnostic methods for diabetes mellitus based on prevalence of retinopathy in a Japanese population: the Hisayama Study

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Abstract

Aims/hypothesis. The aims of this study were to compare the ability of tests measuring fasting plasma glucose, 2-h plasma glucose and HbA_1c levels in predicting specific diabetic retinopathy, and to determine the cut-off level of each measurement for diagnosing diabetes in a Japanese population.
Methods. In a total of 1637 subjects, fasting plasma glucose, 2-h plasma glucose and HbA_1c levels were measured in a 75-g oral glucose tolerance test, and diabetic retinopathy was assessed by ophthalmic examination. We calculated receiver operating characteristic (ROC) curves as well as the prevalence of diabetic retinopathy by deciles of the distribution of these glycaemic measurements.
Results. Of the subjects, 37 (2.3%) had diabetic retinopathy. The prevalence of retinopathy dramatically increased in the tenth decile of each variable. Analysis with ROC curves showed that the optimal cut-off levels for diagnosis of diabetes were 6.4 mmol/l for fasting plasma glucose, 11.1 mmol/l for 2-h plasma glucose, and 5.7% for HbA_1c. The sensitivities for the cut-off point of the three measurements were identical (86.5%), and the specificities were similar (fasting plasma glucose 87.3%; 2-h plasma glucose 89.6%; HbA_1c 90.1%). The area under the ROC curve for 2-h plasma glucose (96.1%) was slightly but not significantly larger than that for fasting plasma glucose (90.0%) and that for HbA_1c (94.5%).
Conclusions/interpretation. Our findings suggest that measuring fasting plasma glucose or HbA_1c is just as useful as measuring 2-h plasma glucose for the diagnosis of diabetes, and that the cut-off point for diagnostic fasting plasma glucose level is lower than that of the current diagnostic criteria.

Keywords Diabetic retinopathy · Fasting plasma glucose · HbA_1c · Receiver operating characteristic curve

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Abbreviations: ADA, American Diabetes Association · FPG, fasting plasma glucose · 2-h PG, 2-hour post-load plasma glucose · NHANES III, the Third National Health and Nutrition Examination Survey · ROC, receiver operating characteristic

Introduction

In 1997 the Expert Committee of the American Diabetes Association (ADA) proposed new revised criteria for diagnosing diabetes [1]. These criteria lowered the diagnostic fasting plasma glucose (FPG) from ≥7.8 mmol/l to ≥7.0 mmol/l, but kept the 2-hour post-load plasma glucose (2-h PG) value at ≥11.1 mmol/l [1]. The WHO subsequently endorsed these recommendations [2]. These diagnostic levels were determined based on the findings of several population-based studies, including those of Pima Indians [3], the Third National Health and Nutrition Examination Survey (NHANES III) participants [1], and Egyptians [4]. However, the sensitivity and tolerability to glucose

load have been reported to vary among ethnic groups [5, 6, 7], and little information has been made available for the Japanese population [8]. In the present article, therefore, we compared the efficacy of tests for FPG, 2-h PG and HbA_1c levels in predicting specific diabetic retinopathy in a Japanese population, and determined the cut-off levels by separating the subjects who were at substantially increased risk of diabetic retinopathy from those who were not.

Subjects and methods

Study population. The Hisayama study is an ongoing prospective cohort study on cardiovascular disease and its risk factors in a community of Hisayama Town adjoining Fukuoka City, a metropolitan area in southern Japan. The enrolment criteria, characteristics of the study population, and overall design of this study have been described in detail in previous studies [9, 10, 11]. As part of the follow-up survey in 1998, we performed a cross-sectional examination, including a 75-g OGTT and ophthalmic examination, of Hisayama residents aged 40 to 79 years. Of a total of 3847 residents in that age group, 1950 subjects (50.7%) consented to participate in the study. After excluding 41 subjects who had already eaten breakfast at the examination, three who were on insulin therapy, 182 who underwent the examination at home, and 87 in whom gradable fundus photographs could not be obtained, a total of 1637 individuals (637 men and 1000 women) successfully completed the 75-g OGTT and ophthalmic examination.

Laboratory measurements. Blood samples were collected from an antecubital vein after an overnight fast for the determination of plasma glucose and HbA_1c levels. After the fasting blood specimen had been taken, the OGTT was performed with a 75-g glucose equivalent carbohydrate load (Trelan G; Shimizu Pharmaceutical, Shimizu, Japan) between 08.00 and 10.30 hours. Subjects receiving oral hypoglycaemic agents omitted their medication until the OGTT. At 120 min after ingestion of the solution, a blood sample was obtained for the determination of post-loading plasma glucose levels. These specimens were analysed within 24 h. Plasma glucose was determined by the glucose–oxidase method, and HbA_1c was measured by a high-pressure lipid chromatographic assay.

Ophthalmic examination and classification of diabetic retinopathy. Each participant underwent comprehensive ophthalmic examination, including stereoscopic fundus examination using indirect ophthalmoscopy, and examination with a slit lamp biomicroscope with a "superfield lens" (Volk, Mentor, Ohio, USA) after pupil dilatation. Fundus photographs (45°) were taken using a Topcon "non-mydriatic" TRC NW-5 fundus camera (Topcon, Tokyo, Japan) and Fujichrome slide film (Sensia II; Fujifilm, Tokyo, Japan). The presence of diabetic retinopathy was determined based on the grading of fundus examinations by indirect ophthalmoscopy, slit lamp, and colour fundus photographs. The photographs were graded by masked photo graders using a modification of the Airlie House classification system: (i) no retinopathic changes; (ii) mild non-proliferative retinopathy; (iii) moderate retinopathy; and (iv) proliferative retinopathy [12, 13, 14, 15]. The degree of diabetic retinopathy was determined according to the grading in the worse eye.

Statistical analysis. The SAS computer package (SAS Institute, Cary, N.C., USA) and Stata version 8.0 (Stata, College Station, Tex., USA) were used to perform all statistical analyses. The sensitivity of a specific glycaemic cut-off point was defined as its ability to correctly identify individuals who have diabetic retinopathy, and its specificity was defined as its ability to correctly identify individuals who do not have diabetic retinopathy. To compare the ability of FPG, 2-h PG and HbA_1c measurements to detect the presence or absence of retinopathy over a range of values, we calculated receiver operating characteristic (ROC) curves and compared the areas beneath them [16, 17]. The diagnostic properties of specific cut-off levels of FPG, 2-h PG and HbA_1c concentrations were defined by maximising the sensitivity and specificity to identify diabetic retinopathy. A two-sided p value of less than 0.05 was considered statistically significant.

Ethical considerations. This study was approved by the Human Ethics Review Committee of Kyushu University Graduate School of Medical Sciences, and was carried out in accordance with the Declaration of Helsinki. Informed consent was obtained from all participants.

Results

Of the study participants, 37 (2.3%) were found to have diabetic retinopathy. Mild non-proliferative retinopathy (category ii), moderate retinopathy (category iii) and proliferative retinopathy (category iv) were found in 27 (1.6%), 5 (0.3%) and 5 (0.3%) subjects respectively. When the subjects were divided according to the ADA fasting glucose criteria, diabetic retinopathy was found in four subjects (0.3%) with an FPG level of <6.1 mmol/l, in nine (5.9%) with an FPG of 6.1 to 6.9 mmol/l, and in 24 (23.1%) with an FPG of ≥7.0 mmol/l (Table 1). Likewise, five subjects (1.8%) with a 2-h PG level of 7.8 to 11.0 mmol/l and 32 (20.5%) with a 2-h PG of ≥11.1 mmol/l had some degree of retinopathy, but there was no subject with retinopathy in the group with a 2-h PG of <7.8 mmol/l.

Figure 1 shows the prevalence of diabetic retinopathy by deciles of the distribution of the FPG, 2-h PG and HbA_1c levels. All three measures of glycaemia were strongly associated with retinopathy, and the prevalence increased dramatically in the tenth decile of each variable, corresponding to an FPG of ≥6.5 mmol/l, a 2-h PG of ≥11.0 mmol/l, and HbA_1c levels of ≥5.8%. The prevalences of retinopathy in the tenth decile of the FPG, 2-h PG and HbA_1c levels were 16%, 20% and 20% respectively, while those in the ninth decile were 3%, 2% and 2% respectively.

To compare the ability of each glycaemic measurement to predict the presence of diabetic retinopathy, we calculated the sensitivity and specificity, and plotted ROC curves. As shown in Figure 2, the area under the ROC curve for 2-h PG was 96.1% (95% CI: 94.4–97.7) and was slightly but not significantly larger than that for FPG (90.0%; 95% CI: 83.8–96.7; p=0.076) and that for HbA_1c (94.5%; 95% CI: 91.6–97.5; p=0.296). The cut-off level defined by the