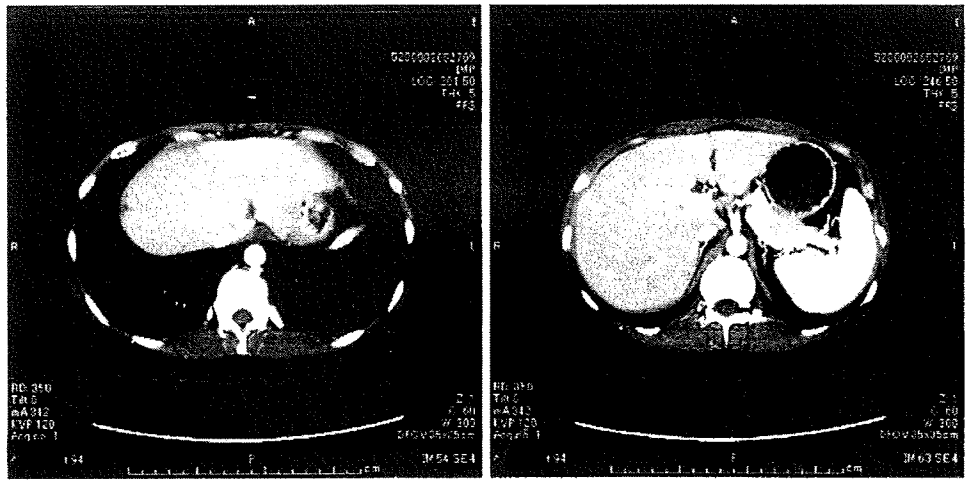

Figure 2. **Computed tomography scan of the upper abdomen 4 weeks after treatment, showing disappearance of most of the low-density areas evident in Fig. 1.**

In the present case, hypocomplementemia during pregnancy might indicate lupus flare. Furthermore, HELLP syndrome involved at least two organs [the liver and hematologic system (DIC)], and met the preliminary criteria for probable CAPS (15). Thus, it was a reasonable decision to increase the dosage of prednisolone in the present case. The treatment was effective for both the clinical symptoms and laboratory abnormalities.

General recommendations for the management of APS in pregnancy and the puerperium have been proposed (5), but the establishment of prophylaxis against HELLP syndrome in patients with APS still remains a controversial issue. Further studies and accumulation of data will be needed in order to clarify the underlying pathogenesis of APS associated with pregnancy leading to a high rate of association with HELLP syndrome.

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特集 新型（豚）インフルエンザ禍からの教訓

5．インフルエンザ流行時における妊婦への対応

高桑 好一*

2009年4月，メキシコで最初の報告がなされた新型インフルエンザは，2009年8月現在，日本も含め世界中で感染が拡大している。従来から，妊婦はインフルエンザ感染のリスク集団と考えられてきたが，新型インフルエンザに関してもそのように捉えられ，妊婦の新型インフルエンザ感染に関するまとまった報告も認められるようになっている。本稿においては，妊婦の免疫的特性とインフルエンザ感染との関連性について説明し，現時点での各種情報をもとに，妊婦が新型インフルエンザに感染した場合の対応，あるいはその予防策について解説を行った。

Key Words：新型インフルエンザ／季節性インフルエンザ／妊娠／ワクチン／抗インフルエンザ薬

I はじめに

「豚インフルエンザ（swine influenza）」としてメキシコで最初の発症が確認された新型インフルエンザは，引き続いて，米国，カナダなどでの発症が報告されたが，2009年8月現在，日本をはじめ世界各国で感染が拡大している。WHO（世界保健機関）は世界的流行であることを宣言し，警戒レベルをフェーズ6に指定している。

妊婦は，新型インフルエンザに関して高リスクグループとみなされているが，その背景には，妊娠現象が母体にとって半同種移植片である胎児胎盤系を体内に宿しており，免疫的に特異的な状況にあることが関与している。本稿においては，新型インフルエンザと妊娠との関連性に関して，最新のデータを交えつつ解説する。

II 妊娠中の免疫的変化とインフルエンザ感染

従来から，季節性インフルエンザウイルスに関して，同ウイルスに対する抗体を有していない妊婦は罹患しやすく，なおかつ重症化しやすいことが指摘されている。Mullooly らは疫学調査により，同年代の非妊娠女性と比較し，妊婦では2.3倍インフルエンザに罹患しやすいことを報告している[1]。また，Neuzil らは，インフルエンザの流行時における妊婦の治療（入院加療）を要する割合について検討した。その結果，分娩終了後の女性と比較し，妊娠中期では1.4倍，妊娠後期では4.7倍であることを観察し，妊婦がインフルエンザ感染した場合，重症化しやすいことを指摘している[2)3)]。

このように，妊婦がインフルエンザに感染しやすく，かつ重症化しやすい背景として，妊婦の免疫的特異性が考慮される。妊孕現象は，半同種移

Management on Pregnant Women during Epidemics of Influenza

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植片である胎児胎盤系が母体からの拒絶を免れ，生着・発育するという現象であり，従来から，妊娠の免疫的維持機構に関する研究，妊娠中の免疫能の変化に関する研究などが行われてきた。初期の研究で，妊娠中に増加する黄体ホルモン，ヒト絨毛性ゴナドトロピン（hCG）などに若干の免疫抑制作用があることが報告されている[4]。また，妊娠中の細胞性免疫能に関する研究が行われ，末梢血リンパ球の機能について低下しているという報告がなされた。すなわち，非特異的マイトージェンに対する反応性の低下[5]，Natural killer（NK）細胞の機能低下[6]などが報告されている。このように，妊娠にともない細胞性免疫能が低下することは以前から指摘されていたが，その後の研究により，液性免疫を誘導する2型ヘルパーT（Th2）細胞が細胞性免疫を誘導する1型ヘルパーT（Th1）細胞に対し優位になることが，妊娠継続にとって有利に作用することが指摘されている[7][8]（図1）。

一般的に，インフルエンザも含めウイルス感染が生じた場合，細胞性免疫誘導物質が感染防御に働くことが指摘されている[9][10]。妊娠中には，細胞性免疫を誘導するTh1細胞系が抑制された状態にあり，またNK細胞活性も低下していることなどから，インフルエンザウイルスに対する感染を起こしやすく，発症した場合，免疫的防御機構の低下から重症化しやすいことが推察される。

Ⅲ　妊婦における新型インフルエンザに関する報告

新型インフルエンザ発症から間もないため，妊婦についてのまとまった報告は認められない状況であったが，2009年5月，CDC（米国疾病対策センター）から，米国における新型インフルエンザ感染の妊婦症例20例が報告された[11]。20例中15例は確定例であり，5例は疑い例であったが，

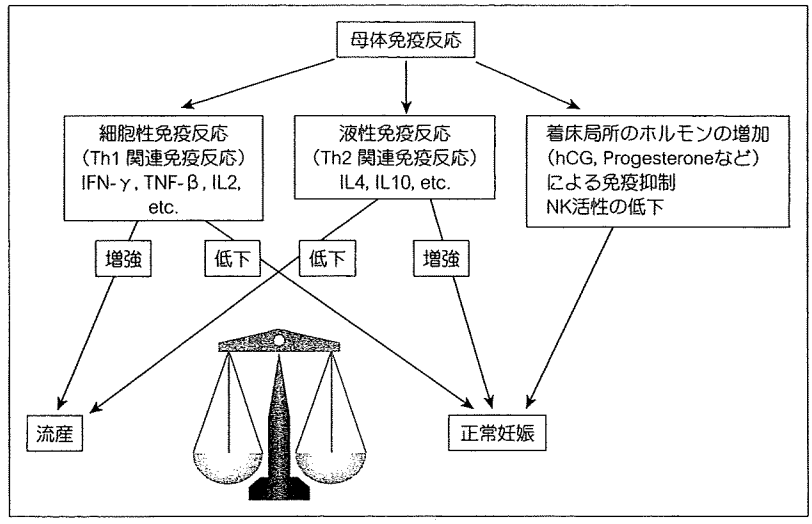

図1　妊娠中の免疫的変化

Th1，Th2バランスについて：正常妊娠では細胞性免疫を誘導する1型ヘルパーT細胞（Th1）が低下し，液性免疫を誘導する2型ヘルパーT細胞（Th2）が優位となることが重要である。（筆者作成）

hCG（ヒト絨毛性ゴナドトロピン）
Th2（2型ヘルパーT）
CDC（米国疾病対策センター）
NK（Natural killer）
Th1（1型ヘルパーT）

3例が入院管理を必要とし，うち1例が発症後約20日で死亡の転帰をとった。

2009年7月には，Jamiesonらが Lancet において，妊娠と新型インフルエンザに関して以下のような詳細な報告を行っている[12]。2009年4月15日～5月18日のあいだに，31例の新型インフルエンザの妊婦感染例および3例の感染疑い例の情報が集積された。年齢は15～42歳であり，初産婦は7例(21%)であった。妊娠の週数については，第1トリメスター（妊娠14週未満）の妊婦が3例（9%），第2トリメスター（妊娠14週～28週）の妊婦が19例(56%)，第3トリメスター（妊娠29週以降）の妊婦が9例（26%）であり，妊娠週数不明例が3例(9%)であった。発症要因として，感染者との濃厚接触があったもの，および外国（メキシコ）への旅行歴があったものが11例（32%）であったが，22例（65%）は推定される発症要因の認められない症例であった。

症状は，咳，鼻汁，咽頭痛，頭痛，筋肉痛など季節性インフルエンザと類似のものであり，新型インフルエンザに特有な症状は認められていない。また，入院を必要とする症例の割合は，非妊娠症例に比較し4.3倍であった。この解析対象例の中で1例の死亡例を認めているが，33歳：妊娠35週の妊婦であり，呼吸困難で入院し，緊急帝王切開術を受けた。その後，挿管管理を必要とし，最終的に肺炎を併発し死亡するに至っている。この症例も含め，2009年4月15日～6月16日のあいだにCDCにより報告された新型インフルエンザによる死亡症例45例のうち，6例が妊婦であり，いずれも合併症を認めない症例であった。

以上がJamiesonらの報告の概要であるが，彼らは，妊婦に対する抗ウイルス薬投与の重要性，新型インフルエンザウイルスに対するワクチンの供給が開始された場合，妊婦に対する接種の重要性を指摘している。

Ⅳ 妊婦に新型インフルエンザ感染が疑われた場合の対応

上述のように，妊婦が新型インフルエンザを発症した場合，重症化することが懸念されており，すみやかな対応が必要である。

表1 リン酸オセルタミビルカプセル(タミフル®)，ザナミビル水和物吸入剤（リレンザ®）の妊婦・産婦・授乳婦等への影響

リン酸オセルタミビルカプセル

1．妊婦または妊娠している可能性のある婦人に投与する場合には，治療上の有益性が危険性を上回ると判断される場合にのみ投与する（妊娠中の投与に関する安全性は確立していない；動物実験〔ラット〕で胎盤通過性が報告されている）。

2．授乳婦に投与する場合には授乳を避けさせる（動物実験〔ラット〕で乳汁中に移行することが報告されている）。

ザナミビル水和物吸入剤

1．妊婦または妊娠している可能性のある婦人に投与する場合には，治療上の有益性が危険性を上回ると判断される場合にのみ投与する（妊娠中の投与に関する安全性は確立していない；動物実験〔ラット，ウサギ〕で胎盤通過性が報告されている）。

2．授乳婦に投与する場合には，授乳を避けさせる（授乳婦に対する安全性は確立していない；動物実験〔ラット〕で乳汁中に移行することが報告されている）。

（文献16より）

2009年8月4日付の日本産科婦人科学会の「妊婦もしくは褥婦に対しての新型インフルエンザ（NIH1）感染に対する対応Q&A」を要約すると，以下のようになる[13]。

1．インフルエンザ様症状が出現した場合の対応について，あらかじめ妊婦と相談しておく。

2．妊婦がインフルエンザ様症状（38℃以上の発熱と急性呼吸器症状）を訴えた場合，産婦人科の直接受診は避けさせ，地域の一般病院への早期受診を勧める。その際には事前に電話連絡をするよう指導しておく。

WHOの勧告では，「新型インフルエンザ感染が疑われる場合には，確認検査を待たずに，できるだけ早期のリン酸オセルタミビル(タミフル®)投与開始を勧めている。したがって，「抗インフルエンザ薬（タミフル®，あるいはザナミビル水和物

吸入剤〔リレンザ® 〕）の早期服用開始（確認検査を待たなくともよい）は，重症化予防に効果があること」を妊婦や家族に伝える。

3．妊婦に新型インフルエンザ感染が確認された場合，抗インフルエンザ薬（タミフル®，あるいはリレンザ® ）の早期服用開始を勧める。

4．妊婦が新型インフルエンザ患者と濃厚接触した場合，抗インフルエンザ薬（タミフル®，あるいはリレンザ® ）の予防的服用を勧める。

5．抗インフルエンザ薬（タミフル®，あるいはリレンザ® ）の胎児に与える影響について，2007年のCDCの報告には，「抗インフルエンザ薬を投与された妊婦および出生した児に有害事象の報告はない」との記載がある。また，わが国の薬剤添付文書には**表1**のような説明がなされており，有益性を考慮して使用する薬剤となっている。

6．抗インフルエンザ薬の予防投与と治療投与の実際（CDCの推奨）

1）タミフル®

予防投与：75mg錠1日1錠。

治療のための投与：75mg錠1日2回。

本邦の「Drugs in Japan」では，治療には上記量を5日間投与，予防には上記量を7～10日間投与となっている。

2）リレンザ®

予防投与：10mgを1日1回吸入。

治療のための投与：10mgを1日2回吸入（計20mg）。

本邦の「Drugs in Japan」では，治療には上記量を5日間吸入，予防には上記量を10日間吸入となっている。尚，予防投与の効果は，これらを使用している期間のみである。

7．授乳について

母乳自体による新型インフルエンザ感染の可能性は，現在のところ知られていない。季節性インフルエンザでは，母乳感染はきわめてまれ。授乳婦への抗インフルエンザ薬の投与は，薬剤の児への潜在的リスクと，母乳栄養の利益を勘案し決定する。抗インフルエンザ薬を服用しながら授乳することは可能であるが，頻繁の手洗い，マスクの着用などを行う。

表2　季節性インフルエンザワクチンの妊婦・産婦・授乳婦等への影響

例：Flu-シリンジ「生研」の場合
妊娠中の接種に関する安全性は確立していないので，妊娠または妊娠している可能性のある婦人には接種しないことを原則とし，予防接種上の有益性が危険性を上回ると判断される場合にのみ接種すること。

（文献16より）

以上が日本産科婦人科学会の提示している，妊婦と新型インフルエンザに関するQ&Aであり，これらの内容を参考として対応することが実際的であると判断される。

V　妊婦に対するインフルエンザワクチンの接種

CDCでは，季節性インフルエンザに関して，妊娠の可能性のある婦人および妊婦に対するワクチンの接種を推奨している[14]。その根拠は，上述のように，妊婦がインフルエンザ感染を起こした場合，重篤化しやすいということである。CDCでは，約2,000人の妊婦がインフルエンザワクチンを受け，出生した児の異常とのあいだに因果関係は認められなかったとの報告を行っている。但し，弱毒生ワクチンは妊婦には使用しないよう指摘されている。

JamiesonはLancetの報告の中で，新型インフルエンザに対するワクチンが応用可能となった場合，妊婦はハイリスク群として優先して使用すべきグループになることを指摘している[12]。

日本産科婦人科学会は，産婦人科診療ガイドラインの中で，「インフルエンザワクチンの母体および胎児への危険性は，妊娠全期間を通じてきわめて低いと説明し，ワクチン接種を希望する妊婦には接種してよい」としている[15]。但し，インフルエンザワクチンの添付文書の説明では**表2**の通り，「妊娠している妊婦には使用しないことを原則とし」との文言がある。このことから，この薬剤説明を提示した上で，同意を得て接種することが現実的であると判断される。新型インフルエンザ

に対するワクチンが応用可能となった場合，妊婦に対する接種の優先順位は高くなることが予測されるが，薬剤に関する説明には留意する必要があるものと考えられる。

Ⅵ おわりに

今年の4月にメキシコから報告された“H1N1 2009インフルエンザウイルス”による新型インフルエンザは，本稿を執筆中の2009年8月現在，世界的流行が進行しており，日本においても厚生労働省から，「大流行の状況」であることが指摘されている。本稿では，2009年8月現在の情報も交え，妊婦と新型インフルエンザとの関連性について解説したが，これらの情報も日々更新されており，厚生労働省，日本産科婦人科学会などから発表されるガイドラインなどを参考とし，診療に臨むことが重要と考えられる。

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周産期医療と inflammatory response

歯周病

高桑好一

はじめに

歯周病は，歯肉炎，歯周炎などを包含する用語であるが，近年，歯周病がさまざまな全身疾患の原因として注目されている。一例として，血管炎であるバージャー病と歯周病の関連性が解析されており，また心筋梗塞などの原因としても注目されている。発症要因として歯周病の原因菌が産生する菌体成分，各種サイトカインなどによる炎症反応が重要視されている。一方，このような炎症反応が原因となり早産，妊娠高血圧症候群，子宮内胎児発育遅延などの異常妊娠が発症することが近年注目されつつあり，また，このような知見に基づき，歯周病のコントロールにより早産，妊娠高血圧症候群などを予防する研究も行われている。本稿においては，歯周病と各種異常妊娠の関連性について概説することとする。

歯周病の分類

歯周病とは歯周組織に発生する疾患の総称とされ，歯垢(プラーク)が主な原因とされるが，非プラーク性の歯周病もある。日本歯周病学会は，アメリカ歯周病学会やヨーロッパ歯周病学会の分類を踏まえ，2006年に歯周病の分類を提唱した。その概要を**表**に提示したが，詳細は文献に記載されている[1]。歯周病の原因菌(歯周病菌)として約500種類の細菌の存在が報告されているが，特に，*Porphylomonas gingivalis*, *Prevotella intermedia*, *Tannerella forsythensis*, *Actinobacillus actinomycetemcomitans*, *Treponema denticola*, *Eikenella corrodens* などの重要性が指摘されている[2]。

表　歯周病の分類(日本歯周病学会，2006年)

Ⅰ．歯肉病変(gingival lesion)：アタッチメントロスを伴わない，歯肉に限局した病変。アタッチメントロスのない歯周組織に初発した場合だけでなく，すでにアタッチメントロスを有する歯周組織に同様の病変が生じた場合でも含むこととする
Ⅱ．歯周炎(periodontitis)：上皮付着の破壊により深部歯周組織に炎症が波及し，アタッチメントロスや骨吸収を生じた疾患
Ⅲ．壊死性歯周疾患(necrotizing periodontal disease)：「壊死性歯肉炎」(歯肉のパンチ状欠損，歯肉出血および疼痛を伴う歯肉壊死を特徴とした感染)と「壊死性歯周炎」(歯肉，歯根膜および歯槽骨の壊死を特徴とした感染)の総称
Ⅳ．歯周組織の膿瘍
Ⅴ．歯肉退縮
Ⅵ．歯周-歯内病変
Ⅶ．咬合性外傷(occlusal trauma)

歯周病と全身疾患

歯周病で認められる歯垢は除菌が困難なbiofilmの状態となっていることが多く[2,3]，全身に細菌を播種させるフォーカスとなり得る。また，細菌の産生する炎症性サイトカインなどにより組織あるいは臓器障害が生ずることが指摘されており，各種疾患と歯周病の関連性が報告されている。具体的にはバージャー病，心筋梗塞，糖尿病などとの関連性が指摘されているが，特にバー

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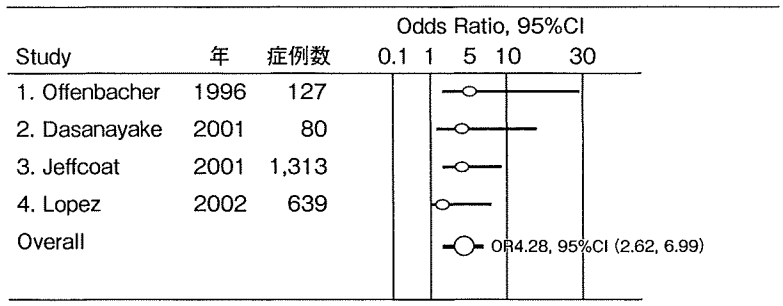

図1　歯周病と早産，低体重児出生との関連性に関する meta-analysis(Khader ら，2005)[6]

ジャー病は強い関連性が認められている。Iwaki ら[4]は 14 例のバージャー病男性患者の口腔内および罹患血管における歯周病菌の検出を行い，14 例中 13 例に歯周病菌が検出されること，特に *Treponema denticola*, *Porphyromonas gingivalis* などが高率に認められることを報告し，バージャー病発症と歯周病との強い因果関係を報告している。彼らは，血管壁に微小膿瘍や巨細胞の浸潤が認められることから，発症機序として喫煙により障害を受けた血管内皮細胞に歯周病菌が感染し血栓形成因子として作用することを推察している。このような機序は，心筋梗塞などでも生じている可能性があり，冠血管においても歯周病菌が存在することが指摘されている[5]。

このような歯周病菌による血管内皮細胞をはじめとする組織障害は，各種周産期異常の発症とも関連する可能性があり，早産，妊娠高血圧症候群などが歯周病を背景として発症することが注目されている。

歯周病と異常妊娠の関連性

1. 歯周病と早産

早産あるいは切迫早産の重要な原因として感染が関与することはすでに明らかとなっているが，歯周病との関連性が解析されている。

Khader ら[6]はメタアナリシスによる解析を試みている。すなわち，信頼性の高い歯周病と早産，低体重児出生との関連性に関する論文[7~10]のデータを検討した。その結果，**図1** に示されたように，対象 2,156 症例に関して，歯周病罹患妊婦群における早産，低体重児出生の発症率が，歯周病非罹患妊婦群に比較し，有意に高率であることを報告している(オッズ比 4.28，95%信頼限界 2.62～6.99)。また，Kornman ら[11]は，妊娠に関連した歯肉炎において，*Prevotella intermedia* が高率に見いだされること，早産に至った妊娠における臍帯血中の抗 *Prevotella intermedia*-IgM 抗体が，満期分娩例に比較し，高率に認められることを報告している。

一方，Heimonen ら[12]は歯周ポケット測定により診断される歯周炎と早産との関連性につき検討を行い，当初関連性がないとの報告を行ったが，ごく最近，歯周炎のみならず歯肉炎，口腔内粘膜炎なども含めた炎症性疾患と早産との関連性を検討し，有病妊婦において早産の発症が有意に高率であることを報告している[13]。

2. 歯周病と妊娠高血圧症候群，子宮内胎児発育遅延

歯周病と妊娠高血圧症候群あるいは子宮内胎児発育遅延との関連性に関する報告もなされている。Contreras ら[14]は 130 例の妊娠高血圧症候群症例，234 例の健常妊婦を比較し，妊娠高血圧症候群症例では 83 例(63.8%)に慢性歯周炎が認められ，健常妊婦における頻度〔89 例(38.0%)〕に比較し有意に高率であることから，妊娠高血圧症候群の発症と慢性歯周炎が関連していることを指

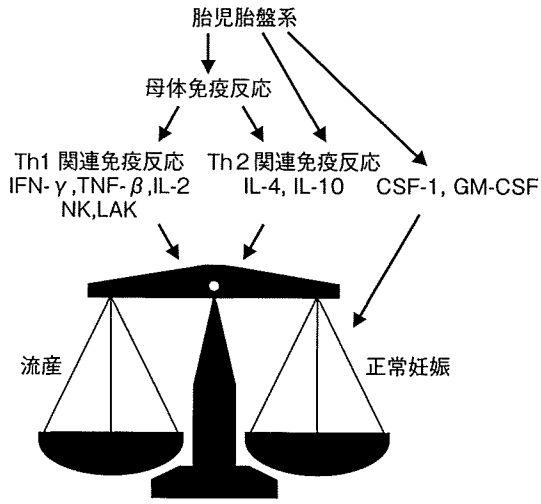

図2　妊孕現象と Th1/Th2 バランス
妊孕現象では細胞性免疫を誘導する1型ヘルパーT細胞(Th1)が低下し，液性免疫を誘導する2型ヘルパーT細胞(Th2)が優位となる

摘している。また，病原菌として，*Porphyromonas gingivalis*，*Tennerella forsythensis*，*Eikenella corrodens* などが有意に高率に観察されることを報告している。

Offenbacher ら[15]は814症例を対象とした解析により，母体の歯周炎と子宮内胎児発育遅延の関連性を指摘している。同じグループである Madianos ら[16]は歯周病菌に対する母体の IgG 抗体，IgM 抗体の有無との関連性を検討し，IgG 抗体の低下が子宮内胎児発育遅延の発症と関連性を有することを報告している。

3. 歯周病による各種異常妊娠の発症機序

歯周病による早産，妊娠高血圧症候群などの発症に関して以下のような機序が推察される。歯周病と全身疾患との関連の項目でも指摘したが，歯周病により，歯周病菌，炎症性サイトカインなどの全身播種が生ずる。ここで重要なことは，妊娠に伴う全身性の免疫的変化であり，1型ヘルパーT細胞免疫反応(Th1，細胞性免疫反応を誘導)に比較し2型ヘルパーT細胞免疫反応(Th2，液性免疫反応を誘導)が優位となり，細胞性免疫能が低下するという点である[17](**図2**)。また妊娠は母体にとって半同種移植片である胎児胎盤系が生着・発育するという自然に成立する移植現象と捉えることができ，母体免疫担当細胞により絨毛組織，胎盤血管などの胎児組織が免疫的障害を受ける可能性が指摘されている。

このような妊娠特有の免疫的環境が増悪因子となり，インターロイキン1β，TNF-αなどの炎症性サイトカインによる血管内皮細胞障害，微小血栓形成などが促進される可能性がある。また，通常，妊娠初期にはらせん動脈が絨毛組織と連結，静脈化し，絨毛間腔の血流増加が生ずるがこのような生理的変化が阻害されることが考えられる。これらの結果，全身性の血管内皮細胞の障害により妊娠高血圧症候群の病態が形成され，絨毛組織，胎盤血管などの障害により胎児発育遅延が生ずることが推察される。

また，歯周病の病原菌そのものの感染により絨毛膜羊膜炎が生じ，一般的な早産の原因として指摘されているように，病原細菌によりプロスタサイクリン E2, F2αなどの子宮収縮促進物質が産生され，その結果早産に至ることが考慮される[18]。

歯周病による各種異常妊娠発症のメカニズムに関する仮説を**図3**に示した。最近では早産発症と炎症性サイトカインの遺伝子多型の関連性が解析され，早産発症に関し感受性を有する多型が報告されている[19]。また，歯周病発症と Fc レセプター遺伝子多型との関連性も指摘されている[20]。これらのことから，歯周病を背景とした各種異常妊娠の発症には遺伝的要因が関与している可能性がある。

歯周病の治療による早産，妊娠高血圧症候群の予防

上述のように，歯周病が各種異常妊娠発症と関連することから，妊婦の歯周病の管理，治療を行うことにより異常妊娠発症の予防を行うという検討がなされている。

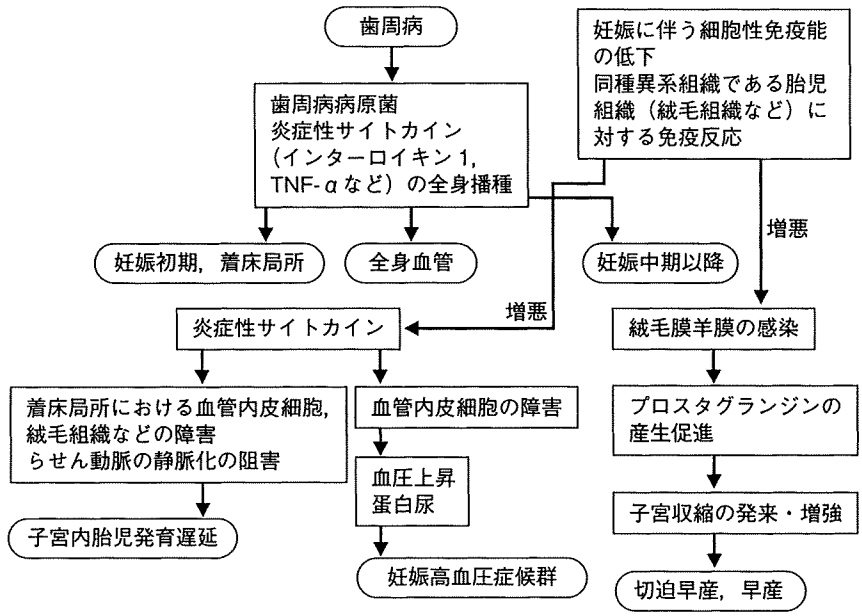

図3　歯周病による異常妊娠発症のメカニズムに関する仮説

López ら[21]は，歯周病治療の早産未熟児出産予防効果に関して以下のようなランダム化試験を行っている。歯科医師により診断がなされた歯肉炎を有する870例の妊婦を対象とし，580例に対しては，歯垢の除去，クロールヘキシジンによる口内洗浄の実施などの治療を実施し，コントロールとした290例に対しては治療を施行せず経過をみた。この結果治療群では早産未熟児の出生率が2.14%であり，コントロール群(6.71%)に比較し有意差をもって低いという結果を得た。一方，Jeffcoat ら[22]は歯周病合併妊婦366名に対し専門の歯科衛生士による口腔内管理を実施し，非施行の723例における妊娠予後と比較した。この結果，管理を実施した群で早産の発症率が有意に低率であることを報告している。このように口腔内の管理により異常妊娠の発症予防の可能性が指摘されているが，多数例を対象とした検討の必要性も同じグループにより指摘されている。

おわりに

歯周病と各種異常妊娠との関連性に関し概説した。多くの報告では，歯周病と早産，妊娠高血圧症候群などの発症との関連性が指摘されているが，これに対し否定的な報告も認められる[23]。人種差，遺伝的背景の差などが関与する可能性もあり，今後さらなる研究が期待される。

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Relationship between single nucleotide polymorphisms in *CYP1A1* and *CYP1B1* genes and the bone mineral density and serum lipid profiles in postmenopausal Japanese women taking hormone therapy

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Abstract

Objective: The genetic variations of the genes encoding cytochrome P-450 enzymes are considered to play an important role in the metabolism of estradiol. The objective of this study was to evaluate the relationships among single nucleotide polymorphisms (SNPs) of cytochrome P-450 genes, lumbar bone mineral density (BMD), and serum lipids and to determine the effects of hormone therapy (HT).

Design: The participants were 124 Japanese women who had been diagnosed with osteopenia or osteoporosis and were taking HT for 12 months. Seven single nucleotide polymorphisms in the *CYP1A1* and *CYP1B1* genes were characterized. Lumbar BMD and the levels of serum lipids were measured before and after HT.

Results: A single nucleotide polymorphism in exon 3 of *CYP1B1* was found to be significantly associated with the effect of HT on BMD and low-density lipoprotein cholesterol both in univariate and multivariate analyses. In the women with the GG genotype of L432V, the responses to HT of BMD and low-density lipoprotein cholesterol markedly decreased. The serum follicle-stimulating hormone level after HT was significantly higher in the women with the GG genotype of L432V.

Conclusions: These results suggest that the L432V polymorphism in the *CYP1B1* gene could therefore be used to predict the effect of HT on lumbar BMD and low-density lipoprotein cholesterol in Japanese women.

Key Words: Single nucleotide polymorphism – *CYP1A1* – *CYP1B1* – Hormone therapy – Bone mineral density – Low-density lipoprotein cholesterol – Follicle-stimulating hormone.

Estrogen plays a significant role in bone and lipid metabolism, and its deficiency after menopause is the main reason for accelerated bone loss and deterioration of the serum lipid profiles, which are preventable by estrogen administration. A number of observational studies have suggested that hormone therapy (HT) reduces the risk of fractures and coronary events in postmenopausal women.[1-4] However, recently published results from randomized clinical trials of HT indicate that this therapy does not slow the progression of coronary atherosclerosis, whereas the reduction in the hip and clinical vertebral fracture rate is significant.[5,6] Our understanding is limited regarding why not all women benefit from such therapy. However, it is still possible that a genetically determined subgroup of the population could benefit from this therapy.

Postmenopausal HT is generally an effective treatment modality to prevent bone loss while also improving the serum lipid profiles; however, individual variations exist.[7-9] Some postmenopausal women respond strongly to HT, whereas approximately 8% who are compliant with this therapy are nonetheless nonresponders.[2] This raises the possibility that some genetic determinants as well as gene-environment interactions might modulate the responses to HT in individual participants.

Individual genetic variability of estradiol metabolism has been described as a significant contributor to the disease susceptibility with variations depending on ethnic background. Among others, the genetic variations of the genes encoding cytochrome P-450 (CYP) enzymes are considered to play an important role in this regard.[10] CYP enzymes play an important role in the production, bioavailability, and degradation of estradiol. A series of polymorphisms and mutations of the CYP enzyme complex have been identified. *CYP1A1* and *CYP1B1* catalyze the hydroxylation of estradiol and several single nucleotide polymorphic sites of those genes have been described.[11,12] Polymorphisms, especially single nucleotide polymorphisms (SNPs) exist in the exon with amino acid changes, thus leading to functionally relevant biochemical consequences that are therefore capable of influencing the responses to HT.

In this study, we attempted to clarify whether SNPs in the exons of the *CYP1A1* and *CYP1B1* genes affected the change in bone mineral density (BMD) and serum lipid profiles in postmenopausal Japanese women during HT.

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METHODS

Design

The participants were 124 Japanese women, ranging in age from 40 to 64 years (49.8 ± 1.0 y, mean ± SEM) who had been diagnosed with osteopenia or osteoporosis and were willing to take HT for 12 months. The diagnoses of osteopenia and osteoporosis were based on the criteria recommended by the Japanese Society of Bone and Mineral Research: a lumbar BMD (L2-4) of less than 80% and less than 70% in younger adults (20-44 y), respectively. In all cases, more than 6 months had elapsed since the last menstrual period, the serum estradiol level was lower than 20 pg/mL, and the serum follicle-stimulating hormone (FSH) level was more than 50 mIU/mL. The exclusion criteria were a history of metabolic disease (including hyperparathyroidism, previously diagnosed osteoporosis, or nontraumatic vertebral fracture on baseline radiograph), chronic disease (uncontrolled hypo- or hyperthyroidism, liver disease, or unstable cardiac disease), cancer or thromboembolic disease, a history of treatment with glucocorticoids for more than 6 months, current HT use or HT use within the past 3 months, or a metabolic or other endocrine disease that could influence lipid metabolism. None of the women smoked or drank alcohol to excess, and none engaged in regular strenuous exercise. Furthermore, none had a history of illness or medical therapy, apart from HT, that might affect bone turnover or lipid metabolism. The women were not genetically related. HT was administered either in a sequential regimen (50 women) consisting of 0.625 mg conjugated equine estrogens for 24 days (days 1-24) and 5 mg medroxyprogesterone acetate for 10 days (days 15-24) or a continuous regimen (74 women) consisting of 0.625 mg conjugated equine estrogens and 2.5 mg medroxyprogesterone acetate for 28 days, according to the woman's preference.

Measures

Bone densitometry

BMD, expressed as the mass per unit area (g/cm^2), was measured in the anteroposterior plane of the lumber spine (L2-4), using dual-energy x-ray absorptiometry with a QDR-2000 analyzer (Hologic Inc., Waltham, MA); absorptiometries were examined by the same observer. The average coefficients of variation of the phantom measurements of bone mineral content, bone area, and BMD during the study period were 1.1%, 0.7%, and 0.6%, respectively. In addition, in the control women, the coefficient of variation of the in vivo precision of BMD between two measurements (mean interval: 2.6 ± 1.2 y) was 0.9%. There was no scanner drift observed during the study period. BMD change (ΔBMD) was expressed as the percentage of BMD change compared with the pretreatment baseline.

Analysis of lipids

After an overnight fast (a minimum 12-h fast), blood was collected from each woman to estimate the lipids and lipoproteins. We measured the total cholesterol (Determiner L-TCN; Kyowa Medex, Tokyo, Japan) and triglyceride (L-type Wako TG-H; Wako Pure Chemical, Osaka, Japan) concentrations by enzymatic methods, and the high-density lipoprotein cholesterol concentration by a homogeneous method (Determiner L HDL-C, Kyowa Medex) using a Hitachi 7450 automated analyzer. Low-density lipoprotein cholesterol was calculated using Friedewald's equation.

Hormones and assays

The serum hormone levels were evaluated after 12 months of HT. Blood samples were drawn in the morning after an overnight fast. The serum was separated immediately and frozen at −80°C for future analysis. The hormone levels were measured using an electrochemiluminescent immunoassay for estradiol and a chemiluminescent immunoassay for luteinizing hormone (LH) and FSH. The hormone fractions were measured in three different batches, and a laboratory batch was also treated to determine the random effect in all hormone analyses. The sensitivity, expressed as the minimal detectable dose, was 11.0 pg/mL, 0.11 mIU/mL, and 0.06 mIU/mL for estradiol, LH, and FSH, respectively. The intra- and interassay coefficients of variation were 1.63% and

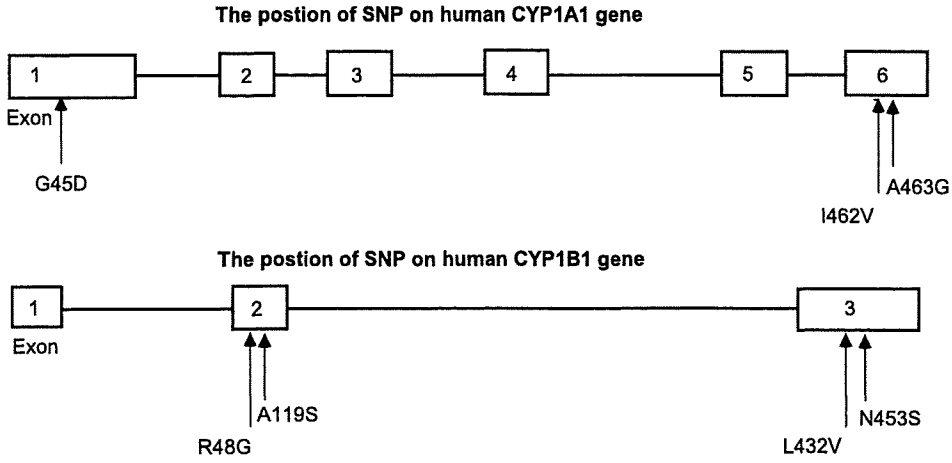

FIG. 1. The position of each single nucleotide polymorphism (SNP) in the *CYP1A1* and *CYP1B1* genes.

TABLE 1. *Genotype and allele frequencies of seven SNPs of CYP gene in Japanese participants*

Gene	Polymorphism	Genotype	Homo (major)	Hetero	Homo (minor)	Allele frequency (major allele)			JSNP ID	dbSNP
						In this study	Sasaki et al	In dbSNP		
CYP1A1	G45D	GA	124 (100%)	0 (0%)	0 (0%)	1.00	—	—	ssj0003953	rs4646422
	I462V	AG	78 (62.9%)	42 (33.9%)	4 (3.2%)	0.80	—	0.902	ssj0007951	rs1048943
	A463G	CG	124 (100%)	0 (0%)	0 (0%)	1.00	—	—	IMS-JST026484	rs2278970
CYP1B1	R48G	CG	90 (72.6%)	16 (12.9%)	18 (1.5%)	0.79	0.68	0.653	ssj0007955	rs10012
	A119S	GT	124 (100%)	0 (0%)	0 (0%)	1.00	0.85	0.648	ssj0007956	rs1056827
	L432V	CG	78 (62.9%)	36 (29.0%)	10 (8.1%)	0.77	0.82	0.592	IMS-JST085313	rs1056836
	N453S	AG	124 (100%)	0 (0%)	0 (0%)	1.00	1.00	0.889	—	rs1800440

SNPs, single nucleotide polymorphisms; CYP, cytochrome P-450; Homo, homozygous; Hetero, heterozygous; JSNP, Japanese Single Nucleotide Polymorphism (database); dbSNP, Single Nucleotide Polymorphism database.

2.24% for estradiol, 3.37% and 3.62% for LH, and 3.50% and 5.28% for FSH.

DNA isolation and genotyping

The peripheral blood samples were collected after informed consent was obtained from each woman. Genomic DNA was extracted from the peripheral blood leukocytes using a DNA purification kit (QIAamp DNA Blood Mini kit; Qiagen, Valencia, CA) according to the manufacturer's instructions. All polymerase chain reactions were performed on a Perkin Elmer GeneAmp 9700 system, and the presence of amplicons was checked on agarose gel. A single nucleotide primer extension assay was performed to analyze SNPs using a SNaPshot Kit (Applied Biosystems, Foster City, CA). The extended primers were analyzed on an ABI 3100 device (Applied Biosystems). The primer sequences for the polymerase chain reactions and primer extension reactions are available in the Japanese Single Nucleotide Polymorphism database. Initial denaturation was performed at 95°C for 2 minutes, followed by 35 cycles each consisting of denaturation at 95°C for 30 seconds, annealing at 60°C, and extension at 72°C for 1 minute, followed by final extension at 72°C for 8 minutes. This study was approved by the Niigata University Human Investigation Committee.

Statistical analysis

Differences in the baseline characteristics, the absolute BMD value, and the serum lipid concentrations among genotypes were tested using an analysis of covariance with age and BMI as covariates. The values of triglycerides were not normally distributed and needed to be log-transformed for the statistical comparisons but, for clarity for presentation, the nontransformed values are presented in the text and tables. To evaluate the relationships between CYP polymorphisms and the change in BMD or serum lipid concentrations during HT, we used repeated-measures analysis of variance. A multiple linear regression model was used to evaluate the simultaneous contributions of different variables. Only those variables that had values of $P < 0.05$ in the univariate analysis were included in the multivariate analyses. All data are expressed as the mean ± SEM. Differences of $P < 0.05$ were considered to indicate statistical significance. All data management and statistical computations were performed with the StatView 4.0 (Abacus Concepts, Berkeley, CA) or the SPSS 10.0 software program (SPSS Inc., Chicago, IL).

RESULTS

In this study, we characterized seven SNPs, three SNPs in the *CYP1A1* gene and four SNPs in the *CYP1B1* gene, from a

TABLE 2. *Baseline characteristics according to the CYP genotypes*

Variables	Genotype of I462V (*CYP1A1*)				Genotype of R48G (*CYP1B1*)				Genotype of L432V (*CYP1B1*)			
	AA (n = 78)	AG (n = 42)	GG (n = 4)	*P*	CC (n = 90)	CG (n = 16)	GG (n = 18)	*P*	CC (n = 78)	CG (n = 36)	GG (n = 10)	*P*
Age, y	50.1 ± 0.8	49.2 ± 0.8	51.3 ± 1.8	0.73	49.6 ± 0.7	51.5 ± 1.4	49.4 ± 1.2	0.53	50.6 ± 0.8	49.0 ± 0.9	47.8 ± 1.4	0.74
Age at menopause, y	47.4 ± 0.6	47.7 ± 0.6	49.0 ± 1.9	0.89	47.6 ± 0.5	46.3 ± 1.6	48.3 ± 0.5	0.46	47.8 ± 0.6	47.2 ± 0.6	46.6 ± 1.9	0.39
Height, cm	154.9 ± 0.6	151.6 ± 2.4	157.8 ± 7.6	0.19	154.9 ± 0.6	154.9 ± 1.5	153.3 ± 0.9	0.51	153.6 ± 0.9	156.1 ± 1.2	158.5 ± 1.1	0.66
Weight, kg	52.5 ± 0.7	51.7 ± 1.0	51.0 ± 6.0	0.76	51.9 ± 0.7	53.4 ± 1.6	52.5 ± 1.6	0.64	52.0 ± 0.7	52.2 ± 1.2	53.2 ± 2.5	0.40
BMI, kg/m^2	21.9 ± 0.26	25.3 ± 3.5	20.3 ± 0.9	0.38	21.6 ± 0.2	22.3 ± 0.6	22.4 ± 0.6	0.34	23.7 ± 1.9	21.7 ± 0.4	22.1 ± 0.7	0.74
L2-4 BMD, g/cm^3	0.76 ± 0.02	0.76 ± 0.02	0.79 ± 0.08	0.96	0.76 ± 0.02	0.79 ± 0.05	0.75 ± 0.05	0.78	0.77 ± 0.02	0.76 ± 0.02	0.78 ± 0.07	0.23
TC, mg/dL	224.4 ± 4.2	227.2 ± 6.5	231.0 ± 13.5	0.89	223.8 ± 3.9	224.9 ± 8.1	234.5 ± 11.8	0.54	226.2 ± 4.3	228.6 ± 6.8	216.1 ± 10.9	0.65
LDL-C, mg/dL	132.6 ± 4.7	137.3 ± 5.9	132.5 ± 7.2	0.82	130.8 ± 3.8	140.1 ± 0.8	147.3 ± 13.0	0.24	136.1 ± 4.8	135.2 ± 6.2	123.9 ± 10.6	0.60
HDL-C, mg/dL	67.6 ± 1.9	67.0 ± 2.9	77.0 ± 6.8	0.51	67.5 ± 1.8	67.0 ± 2.9	69.6 ± 6.1	0.88	67.4 ± 1.9	68.0 ± 3.1	67.0 ± 6.1	0.98
TGs, mg/dL	122.9 ± 8.8	115.8 ± 11.3	84.8 ± 12.5	0.55	121.2 ± 8.4	114.4 ± 14.3	102.1 ± 11.8	0.60	115.7 ± 9.0	125.3 ± 11.4	125.7 ± 24.5	0.76

CYP, cytochrome P-450; BMI, bone mass index; BMD, bone mineral density; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TGs, triglycerides.
Data are presented as mean ± SE.

total of 248 chromosomes from 124 postmenopausal Japanese women. Figure 1 indicates the location of each SNP analyzed in this study. All SNPs exist within the exon, thus resulting in amino acid substitution.

Although the genotypic distribution of I462V in the *CYP1A1* gene was in Hardy-Weinberg equilibrium, those of R48G and L432V in the *CYP1B1* gene were observed to deviate from Hardy-Weinberg equilibrium. The frequencies of the variant SNP alleles ranged from 19% to 23%. There were no variant alleles in four SNPs (G45D, A463G, A119S, and N453S [*CYP1B1*]) in the population analyzed in this study (Table 1). In addition, no significant differences were observed in the baseline characteristics with any genotypes tested in this study (Table 2). No significant differences were observed in either the baseline characteristics or the response to HT (data not shown).

To test whether these three exon SNPs might be involved in the response to HT, the percentage of changes in the lumbar BMD and the serum lipid profiles after HT were compared according to each genotype of the CYP genes (Table 3). The genotype L432V in the *CYP1B1* gene demonstrated significant associations with lumbar BMD and low-density lipoprotein cholesterol (LDL-C) responses after 12 months of HT. Neither the genotype I462V (*CYP1A1*) nor R48G (*CYP1B1*) demonstrated a significant association with the lumbar BMD or the serum lipid responses. The mean change in the BMD of all women after 12 months of treatment was 2.3 ± 0.5%. Although the absolute value of the BMD did not show any significantly difference among the different genotype groups, the participants with the homozygous (variant) genotype (GG) of L432V showed significantly less BMD change (−3.7 ± 2.4%) than those with the heterozygous (CG; 1.8 ± 1.0%) and homozygous (wild type) (CC; 3.4 ± 0.6%) genotypes. The serum LDL-C level of all women decreased (−13.5 ± 2.7%) after 12 months of treatment. In the women with the heterozygous (CG) and homozygous (CC; wild type) genotypes of L432V, the LDL-C level decreased, whereas that in women with the homozygous (variant) genotype (GG) of L432V inversely increased (11.1 ± 3.5%) after 12 months of treatment.

In the univariate analysis, some factors, other than the L432V polymorphism, significantly influenced the lumbar BMD and LDL-C responses. For example, with older age and a higher baseline BMD, there was less increase in BMD response to HT, and with a higher baseline LDL-C, there was less decrease in LDL-C. Body weight and BMI did not influence those responses to HT.

Finally, the effect of the L432V genotype on the responses of lumbar BMD and LDL-C were maintained after adjustment for the significant variables in the univariate analysis (Table 4). This confirms the independent effect of the L432V polymorphism in the *CYP1B1* gene on the response to HT.

To evaluate the relationship between the L432V SNP and the circulating hormone levels, serum estradiol, LH, and FSH levels after 12 months of HT were compared among the genotypes of L432V. Although the serum levels of estradiol and LH did not show any significant differences, the serum

TABLE 3. *Changes in the lumbar BMD and serum lipids after HT according to the CYP genotypes*

	% change (absolute value)											
	Genotype of I462V (*CYP1A1*)				Genotype of R48G (*CYP1B1*)				Genotype of L432V (*CYP1B1*)			
Variables	AA (n = 78)	AG (n = 42)	GG (n = 4)	*P*	CC (n = 90)	CG (n = 16)	GG (n = 18)	*P*	CC (n = 78)	CG (n = 36)	GG (n = 10)	*P*
L2-4 BMD, g/cm^2	2.4 ± 0.6	2.1 ± 1.2	3.9 ± 1.5	0.833	2.4 ± 0.6	2.6 ± 1.4	1.7 ± 1.2	0.872	3.4 ± 0.6	1.8 ± 1.0	−3.7 ± 2.4	0.002
	(0.78 ± 0.02)	(0.77 ± 0.02)	(0.81 ± 0.09)		(0.78 ± 0.01)	(0.79 ± 0.04)	(0.76 ± 0.04)		(0.78 ± 0.02)	(0.77 ± 0.02)	(0.74 ± 0.06)	
TC, mg/dL	−3.8 ± 2.3	−4.8 ± 1.9	−6.3 ± 6.6	0.9330	−4.5 ± 2.0	−4.0 ± 3.3	−3.1 ± 4.2	0.953	−4.2 ± 1.7	−9.4 ± 3.5	5.5 ± 4.4	0.058
	(212.0 ± 4.6)	(211.3 ± 5.5)	(213.5 ± 5.3)		(212.0 ± 3.5)	(213.4 ± 6.2)	(221.6 ± 8.7)		(210.3 ± 4.5)	(206.1 ± 4.7)	(226.1 ± 11.4)	
LDL-C, mg/dL	−11.0 ± 4.0	−17.4 ± 3.2	−16.6 ± 6.1	0.455	−13.5 ± 3.2	−6.3 ± 6.5	−20.5 ± 7.4	0.302	−15.6 ± 3.8	−18.0 ± 4.2	11.1 ± 3.5	0.002
	(116.8 ± 5.0)	(118.3 ± 3.2)	(114.0 ± 4.4)		(114.6 ± 4.3)	(125.0 ± 5.4)	(124.6 ± 6.9)		(115.6 ± 4.9)	(114.4 ± 4.3)	(140.0 ± 10.9)	
HDL-C, mg/dL	3.0 ± 2.9	8.7 ± 3.5	3.0 ± 3.1	0.408	4.5 ± 2.8	5.3 ± 2.5	7.2 ± 4.1	0.894	4.5 ± 1.9	3.0 ± 6.0	−2.5 ± 4.1	0.827
	(71.3 ± 2.0)	(71.8 ± 2.8)	(78.8 ± 4.0)		(70.8 ± 1.7)	(71.9 ± 3.3)	(75.8 ± 5.2)		(71.5 ± 2.0)	(70.9 ± 2.6)	(68.4 ± 7.4)	
TGs, mg/dL	15.7 ± 6.7	14.5 ± 8.6	38.3 ± 25.8	0.698	19.8 ± 6.5	1.9 ± 14.4	10.7 ± 6.4	0.252	21.2 ± 6.5	5.9 ± 10.3	16.8 ± 13.5	0.357
	(129.6 ± 7.0)	(115.7 ± 10.3)	(111.5 ± 38.3)		(127.9 ± 6.7)	(113.1 ± 11.3)	(115.0 ± 16.3)		(122.1 ± 6.8)	(124.5 ± 11.5)	(137.0 ± 19.9)	

BMD, bone mineral density; HT, hormone therapy, CYP, cytochrome P-450; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol. HDL-C, high-density lipoprotein cholesterol; TGs, triglycerides.
Data are presented as mean ± SE.

level of FSH showed significant differences among the L432V genotypes (Table 5). Compared with the women with the CC genotype (wild type, homozygous), women with the GG genotype (mutant, homozygous) had a significantly higher level of FSH ($P = 0.006$) after 12 months of HT.

DISCUSSION

Variations in the estrogen-metabolizing genes, such as *CYP1A1*, *CYP1B1*, *CYP17*, and *CYP19*, and catechol-*O*-methyltransferase genes have been reported regarding the susceptibility of women to breast cancer, and such variations were also found to influence the clinical course.[13,14] Furthermore, the SNPs of these genes have been evaluated in women using a variety of factors, such as the age at menarche and natural menopause,[15] breast density,[16] and plasma estrogen levels.[17,18]

Both the *CYP1A1* and *CYP1B1* loci appear to play a prominent role within the genes involved in estrogen metabolism. CYP1A1 catalyzes the C2-, C6-, and C15-α hydroxylation, whereas *CYP1B1* catalyzes the C4-hydroxylation of estradiol. Various polymorphic sites of the *CYP1A1* and *CYP1B1* genes have been described on either introns or exons.

In this study, women with a homozygous variant allele of L432V showed significantly poor responses to HT. The genotype frequency distributions of L432V in the *CYP1B1* gene were found to deviate from the Hardy-Weinberg equilibrium because of a variant homozygote excess. This variant in the *CYP1B1* gene is thus possibly an important candidate for an SNP predisposing to the development of either postmenopausal osteopenia or osteoporosis, although the baseline BMD did not significantly differ between the different genotypes in this study.

The catalytic activities of variant enzymes, especially the nucleotide changes in exon 2 (A119S polymorphism) and exon 3 (L432V polymorphism) of the *CYP1B1* gene, have been reported to be two- to fourfold higher than those of wild-type enzymes.[19-22] A significant decrease in the estradiol levels in postmenopausal women with the L432V variant homozygous genotype has been also reported.[18] In this study, significantly higher serum FSH levels during HT in women with an L432V variant genotype were observed, even though there was no significant difference in the serum estradiol level. Although several investigators have shown estradiol to be a predictor of bone loss,[23,24] there is a conflicting report in which there was no significant correlation of estradiol levels with BMD.[25] The peripheral levels of estradiol may not necessarily represent the estradiol levels in target tissues.[26] Thomsen et al[27] reported a strong correlation between the decrease in FSH and the change in BMD, whereas the association between BMD and the estradiol level was less clear. They also reported that women who have a favorable response in BMD during HT also tend to show a favorable change in the lipid profile, and this association is most likely driven by a common response of FSH to exogenous estrogen therapy. Therefore, the L432V variant that corresponds to the hyperactivity of *CYP1B1* accelerates estradiol metabolism, thus leading to higher serum FSH levels and thus may possibly affect the response to HT regarding the lumbar BMD and serum lipid profiles.

There are some limitations to our study. Gonadotropins are known to be secreted in an episodic fashion. The pulse amplitude of FSH in postmenopausal women with HT has been reported to be 5.7 ± 1.0 mIU/mL. Therefore, the validity of the gonadotropin determinations based on a single blood measurement may be questioned. In addition, the number of the L432V variants in this study was limited. Additional studies are therefore necessary to clarify the precise mechanisms by which the *CYP1B1* polymorphisms modulate the responsiveness of BMD and LDL-C to HT.

TABLE 4. *Baseline variables as predictors of the percent change in the lumbar BMD and serum LDL-C after HT: multivariate regression analysis*

Variables	Correlation coefficient *r*	*P*
BMD		
Age	0.130	0.107
Baseline BMD	−0.416	<0.001
L432V (CYP1B1) genotype	0.273	<0.001
LDL-C		
Baseline LDL-C	−0.501	<0.001
L432V (CYP1B1) genotype	0.182	0.039

BMD, bone mineral density; LDL-C, low-density lipoprotein cholesterol; HT, hormone therapy.

TABLE 5. *Serum hormone levels at 12 months after HT according to the genotype of L432V in the CYP1B1 gene*

	Genotype			
	CC (n = 20)	CG (n = 20)	GG (n = 10)	*P*
Estradiol, pg/mL	71.3 ± 7.3	74.3 ± 14.3	69.9 ± 16.8	0.971
LH, mIU/mL	11.2 ± 2.6	15.5 ± 3.2	16.2 ± 6.2	0.560
FSH, mIU/mL	9.4 ± 1.1	15.7 ± 3.3	24.1 ± 6.4	0.021

HT, hormone therapy; LH, luteinizing hormone; FSH, follicle-stimulating hormone.
Data are presented as mean ± SE. Controlling for age, date of blood draw, time of blood draw, fasting status, body mass index, and laboratory batch.

CONCLUSIONS

In summary, our genetic analyses of the genes *CYP1A1* and *CYP1B1* suggest that the L432V SNP in the *CYP1B1* gene might act as a marker of the drug response. An analysis of the *CYP1B1* gene SNPs might therefore prove to be useful in appropriately selecting HT for the management of either osteopenia or hyperlipidemia in Japanese postmenopausal women.

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Progressive Renal Tubular Dysfunction Associated with Long-Term Use of Tenofovir DF

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Abstract

It became evident that tenofovir DF (TDF) causes a modest and gradual decline in GFR, however, the impact of long-term use of TDF on tubular function has not been fully evaluated. In 40 patients treated with TDF and 23 patients treated with other NRTIs, urine β_2-microglobulin (U-BMG), percentage tubular reabsorption of phosphate (%TRP), alkaline phosphatase (ALP), serum creatinine, and calculated GFR were prospectively measured for 96 weeks. In patients receiving TDF, median U-BMG rose from 188 μg/liter at baseline to 555 μg/liter at week 96 ($p = 0.02$), median %TRP declined from 94% at baseline to 90% at week 96 ($p = 0.002$), median ALP ratio compared with baseline persistently increased from 1 to 1.278 at week 96 ($p = 0.001$), and serum creatinine showed significant but minimal change from 0.64 mg/dl to 0.74 mg/dl at week 96 ($p = 0.02$). The GFR level declined minimally but significantly in TDF-receiving patients (–17 ml/min/1.73 m^2), whereas it did not change in other NRTI-receiving patients [+3 ml/min/1.73 m^2; mixed models analysis of variance (MMANOVA) $p = 0.03$ for overall change from baseline to week 96]. U-BMG, %TRP, ALP, or serum creatinine did not change significantly in other NRTI-receiving patients during the observation period. In five patients with marked changes in U-BMG (>10,000 μg/liter) and %TRP (<80%), both U-BMG and %TRP immediately recovered in all patients after discontinuing TDF, whereas GFR levels did not fully recover for 6 months in three patients. Prolonged treatment with TDF caused progressive renal tubular dysfunction as well as a modest decline in GFR. If U-BMG levels >10,000 μg/liter and %TRP values <80% are observed, discontinuing TDF may be beneficial.

Introduction

TENOFOVIR DISOPROXIL FUMARATE (TDF), a nucleotide analogue of adenosine 5′-monophosphate, is one of the most widely used antiretroviral agents for HIV-1-infected patients. Although clinical trials have concluded that TDF-associated renal toxicity is rare and reversible,[1–3] it is evident that long-term administration of TDF causes a gradual decrease in glomerular filtration rate (GFR).[4–7] Furthermore, a growing number of case reports suggested that TDF-associated renal toxicity is mainly caused by proximal tubular injury.[8–11] TDF is excreted via renal proximal tubular transporters.[12,13] Adefovir and cidofovir, both nucleotide analogues, have been reported to cause human renal toxicity via mitochondrial injury in renal tubular epithelial cells.[14] Nevertheless, the impact of the long-term use of TDF on proximal tubular function has not been fully evaluated.

Materials and Methods

This study was conducted prospectively from May 2004 to May 2007. Among 164 HIV-1-infected patients who were registered in Ogikubo Hospital, 110 patients were treated with antiretroviral drugs. Of 110 treated patients, 63 patients who could come to Ogikubo Hospital regularly to have regular blood and urine sampling with informed consents were enrolled in this study. Exclusion criteria were a moderately low level of calculated GFR (<80 ml/min/1.73 m^2). Of 63 enrolled patients, 40 patients were treated with a TDF-based regimen and 23 patients were treated with another NRTI-based regimen. The characteristics of the sample population are shown in Table 1. Of the 40 patients who received TDF, 32 ART-experienced patients simply switched from d4T to TDF to avoid future risk of lipodystrophy or other d4T-related adverse effects. In TDF-receiving patients ($n = 40$), combined NRTIs were as follows: 34 patients with lamivudine (3TC) or emtricitabine (FTC), 4 patients with abacavir (ABC), and 2 patients with didanosine (ddI). In other NRTI-receiving patients ($p = 23$), combinations of two NRTIs were as follows: 10 patients with zidovudine (ZDV) + 3TC, 10 patients with stavudine (d4T) + 3TC, 2 patients with d4T + ddI, and 1 patient with d4T + ABC. Informed consent was obtained from all enrolled patients.

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TABLE 1. CHARACTERISTICS OF SAMPLE POPULATION

	TDF	*Other NRTI*
Number of patients	40	23
Sex male	40 (100%)	23 (100%)
Median of age (range)	35 (27–66)	32 (22–68)
Median of CD4 (cell/mm^3)	376 (69–1243)	224 (12–748)
Median of HIV RNA (copies/ml)	33 (<50–100,000)	18,000 (<50–100,000)
History of HAART	Naive 8 Experienced 32	Naive 9 Experienced 14
Underlying antiretrovirals		
Efavirenz	14 (35%)	6 (26%)
Nevirapine	3 (8%)	0 (0%)
Atazanavir/ritonavir	16 (40%)	6 (26%)
Lopinavir/ritonavir	5 (13%)	3 (13%)
Nelfinavir	2 (5%)	6 (26%)
Dual therapy	0 (0%)	2 (9%)
Route of HIV-1 infection		
Contaminated blood products	36 (90%)	17 (74%)
Sexual transmission	4 (10%)	6 (26%)
Underlying disease		
Diabetes mellitus	6 (15%)	2 (9%)
Indinavir-associated renal atrophy	2 (5%)	0 (0%)
Pretreatment with indinavir	7 (18%)	7 (0%)

Laboratory testing

Urine β_2-microglobulin (U-BMG), %TRP, alkaline phosphatase (ALP), serum phosphorus, serum uric acid, serum creatinine, and GFR were prospectively measured along with a urinalysis performed every 4–12 weeks, from baseline to 96 weeks, in 40 patients treated with TDF. In 23 patients treated with other NRTIs, serum creatinine, GFR, and ALP were prospectively measured every 3 months, while U-BMG and %TRP were measured every 12 months for 2 years in 17 patients during the same period. U-BMG was determined using a spot urine sample. %TRP was calculated using the following formula: %TRP = [1– (urine phosphorus/serum phosphorus × serum creatinine/urine creatinine)] × 100. Urine phosphorus and urine creatinine were measured on the spot urine sample and serum creatinine and serum phosphorus levels were obtained from blood samples on the same day. GFR was calculated based on the simplified modification of diet in renal disease (MDRD) equation, which is described in the National Kidney Foundation's Kidney Disease Outcomes Quality Initiative.[15] In urinalysis, urine glucose and urine protein were evaluated in accordance with the color chart of the dipsticks (Labsticks; Bayer Medical Corp., CA). Renal tubular epithelial cells were counted with high-power fields (HPF; 400× objective), and granular casts were counted with low-power fields (LPF; 100× objective) from urine sediments.

Statistical analyses

Changes over time within groups were assessed using the Wilcoxon signed rank test, and levels of renal parameters between two groups at week 96 were compared using the Mann–Whitney *U* test. Moreover, mixed models analysis of variance (MMANOVA) was used to assess the overall pattern of changes in GFR from baseline to week 96 in the total sample population.[16] MMANOVA allowing for the influence of TDF/other NRTI assignment, naive/experienced assignment, baseline U-BMG level, and a potential interaction between TDF/other NRTI assignment and naive/experienced assignment was applied to adjust the significance level for the change on GFR. All analyses were performed using SAS release 8.02 (SAS Institute, Cary, NC).

Results

Renal parameters

In TDF-receiving patients, 3/40 (8%) patients discontinued TDF before week 96 due to a progressive decline of GFR and tubular dysfunction, whereas there was no patient who discontinued antiretrovirals in other NRTI-receiving patients.

The median [interquartile range (IQR)] of U-BMG was significantly elevated from 188 (134–359) μg/liter at baseline to 555 (229–1425) μg/liter at week 96 ($p = 0.02$) in TDF-receiving patients (Fig. 1a). Highly elevated U-BMG (>10,000 μg/liter) was observed in 3 of 40 (8%) patients by week 96, and moderately elevated U-BMG (1000–10,000 μg/liter) was observed in 15 of 40 (38%) patients by week 96. In contrast, in other NRTI-receiving patients ($n = 17$), U-BMG did not change significantly during the study period [from 170 (96–217) μg/liter at the initial point of the study to 150 (81–307) μg/liter at the end of the study; $p = 0.37$], and there was no patient with a moderate or marked elevation of U-BMG (≥1000 μg/liter).

The median (IQR) of %TRP showed a significant decline from 94 (92–96)% at baseline to 90 (89–95)% at week 96 in TDF-receiving patients ($p = 0.04$; Fig. 1a), whereas there was no significant decline of %TRP in the other NRTI group [$n = 17$; from 96 (95–97)% at the initial point to 94 (91–96)% at week 96 ($p = 0.33$)]. A marked decline in %TRP (<80%) was observed in 3/40 (8%) patients, and a moderate decline (80–90%) was observed in 18/40 (45%) patients at week 96, whereas there was no patient with a moderate or marked decline (%TRP <90%) in the other NRTI group. Comparing the patients who had mildly decreased %TRP (%TRP <90% on two occasions)

with those with normal %TRP (%TRP ≥90%), the former had significantly higher levels of median serum creatinine [0.79 (0.74–0.91) mg/dl vs. 0.63 (0.56–0.70) mg/dl, $p=0.002$] and significantly lower levels of median GFR [118 (106–136) ml/min/1.73 m^2 vs. 160 (139–177) ml/min/1.73 m^2, $p=0.001$] at week 96.

The median (IQR) of MDRD-GFR declined gradually from 150 (126–165) ml/min/1.73 m^2 at baseline to 136 (116–157) ml/min/1.73 m^2 at week 96 ($p=0.02$) in TDF-receiving patients, whereas it did not change in other NRTI-receiving patients ($n=23$) [from 129 (112–138) ml/min/1.73 m^2 at baseline to 136 (124–145) ml/min/1.73 m^2 at week 96 ($p=0.39$); Fig. 1b]. In using the Cockcroft-Gault equation, the median (IQR) of GFR also declined from 138 (112–155) ml/min/1.73 m^2 at baseline to 127 (105–148) ml/min/1.73 m^2 at week 96 ($p=0.02$), whereas it did not significantly change in other NRTI-receiving patients [from 129 (117–143) ml/min/1.73 m^2 at baseline to 135 (116–155) ml/min/1.73 m^2 at week 96 ($p=0.65$)]. The change in MDRD-GFR over time was reassessed using MMANOVA. In using MMANOVA, GFR declined significantly in TDF-receiving patients (–17 ml/min/1.73 m^2, $p=0.04$), whereas it did not change in other NRTI-receiving patients (+3 ml/min/1.73 m^2, $p=0.43$). The overall difference between the two treatment group was statistically significant (MMANOVA, $p=0.03$). GFR change was not significantly influenced by previous administration of HAART (MMANOVA, $p=0.07$) or baseline U-BMG levels (MMANOVA, $p=0.28$). There was no significant interaction between TDF/other NRTI assignment and naive/experience assignment (MMAOVA, $p=0.73$). The median (IQR) of serum creatinine increased from 0.64 (0.59–0.75) mg/dl at baseline to 0.74 (0.64–0.80) mg/dl at week 96 ($p=0.02$), whereas it did not change in other NRTI-receiving patients [$n=23$; from 0.73 (0.68–0.83) mg/dl at baseline to 0.70 (0.66–0.78) mg/dl at week 96 ($p=0.14$), respectively].

The median (IQR) of ALP persistently and significantly rose during the study period in the TDF group [from 289 (261–382) IU/liter at baseline to 355 (280–421) IU/liter at week 96 ($p=0.001$)], whereas it did not change significantly in other NRTI groups [from 172 (138–250) IU/liter at baseline to 180 (148–247) IU/liter at week 96 ($p=0.98$)]. In comparing the ALP ratio (relative to baseline), the median (IQR) ALP ratio in patients receiving TDF was significantly higher than in patients receiving other NRTIs [1.278 (1.059–1.354) vs. 1.003 (0.876–1.098) ($p=0.02$); Fig. 1c]. Even in patients receiving TDF, serum phosphorus and serum uric acid were not significantly decreased during the study period. The median (IQR) serum phosphorus level was 3.4 (2.9–3.6) mg/dl at baseline and 3.0 (2.7–3.4) mg/dl at week 96 ($p=0.20$), and serum uric acid was 6.1 (5.0–7.0) mg/dl at baseline and 5.5 (4.9–6.6) mg/dl at week 96 ($p=0.08$).

In TDF-receiving patients, a reduction in GFR level was associated with U-BMG levels. GFR significantly decreased in patients with higher U-BMG (≥1000 μg/liter) in two or more occasions from 132 (124–159) ml/min/1.73 m^2 at baseline to 118 (104–151) ml/min/1.73 m^2 at week 96 ($p=0.01$), whereas it did not decrease in the other patients [from 155 (134–172) mg/dl at baseline to 143 (133–164) mg/dl ($p=0.59$)]. In comparing GFR levels at week 96 between the patients with higher U-BMG (≥1000 μg/liter) on two or more occasions and those with lower U-BMG (<1000 μg/liter), the former level was significantly lower than the latter [118 (104–151) ml/min/1.73 m^2 vs. 143 (133–164) ml/min/1.73 m^2 ($p=0.04$)].

In urinalysis, the ratio of the patients with positive urine protein did not significantly increase in both TDF-receiving patients and other NRTI-receiving patients [19% at baseline and 26% at week 96 ($p=0.84$) in TDF-receiving patients and 5% at baseline and 5% at week 96 in other NRTI-receiving patients ($p=0.86$), respectively]. There was no statistical difference in the ratio of positive urine protein at week 96 between the two groups. A ratio of the patients with positive urine glucose did not significantly change in both TDF-receiving patients and other NRTI-receiving patients [19% at baseline and 26% at week 96 in TDF-receiving patients ($p=0.84$) and 5% at baseline and 5% at week 96 in other NRTI-receiving patients, respectively]. Granular cast was observed in 5% at baseline and 6% at week 96 in TDF-receiving patients, and 0% at baseline and 0% at week 96 in other NRTI-receiving patients. There was no significant difference between the two groups at baseline or week 96 ($p=0.77$ and 0.85, respectively). Renal tubular epithelial cells were observed in 17% at baseline and 8% at week 96 in TDF-receiving patients ($p=0.46$), and 5% at baseline and 9% at week 96 in other NRTI-receiving patients. There was no significant difference between the two groups at baseline or week 96 ($p=0.29$ and 0.43, respectively). Among the five TDF-receiving patients with rapid deterioration of U-BMG and %TRP, granular casts were observed in only two patients and renal tubular epithelial cells were observed in three patients.

Severe TDF-associated renal toxicity and its recovery after discontinuation of TDF

In this study, severe renal toxicity was observed in five TDF-receiving patients (Table 2), whereas neither reduction of GFR nor tubular dysfunction was observed in other NRTI-receiving patients. Among these five patients, three patients (Patients 1–3 in Table 2) showed TDF-associated renal toxicity during the study period, and they discontinued TDF. The other two patients had acute renal failure after the study period (Patients 4 and 5). An extremely abnormal value of U-BMG (>10,000 μg/liter) and %TRP (<80%) were observed in all five patients, but both of them recovered to baseline levels immediately after TDF was discontinued in all cases. In three of five patients, GFR levels rapidly declined from a normal level (>90 ml/min/1.73 m^2) to a mildly decreased level (60–89 ml/min/1.73 m^2), and in the other two patients, it declined from normal to a moderately decreased level (30–59 ml/min/1.73 m^2). In three patients (Patient 1, 2, and 4), the GFR level did not fully recover for 6 months after discontinuation of TDF (Table 2).

No association between TDF-associated renal toxicity and low CD4 cell count

Among TDF-receiving patients, urine-β_2-microglobulin, %TRP, ALP, GFR, and serum creatinine were compared between patients with low CD4 cell counts at baseline (<200/μl; $n=11$) and patients with normal CD4 cell counts at baseline (≥200/μl; $n=29$). In the 11 patients with low CD4 cell count <200, U-BMG at baseline and week 96 was 307 (235–455) μg/liter and 411 (262–711) μg/liter; %TRP was 94