表 3 MTCT rate by breast and bottle feeding (ROC)

	Breast feeding	Bottle feeding	Total
Infants(n)	69	11	80
HIV (+) infants	26	2	28
MTCT rate	37.7%* (26/69)	18.2%* (2/11)	35.0% (28/80)

* : $p<0.05$ (Shukang Xiong)

については European Collaborative Study (2005) の報告によれば HAART 実施例における母子感染率を更に 0.5-1.0 % 低下させる効果があるとのことである。経済的に余裕のある国ではこの事実は無視出来ないが，一方米国マイアミ大学医療センターでは HIV viral load が 1,000 copies/ml 未満であれば経膣分娩を許可しており (CDC Report, 2006)，2.5% 以下の母子感染率を得ている。HIV 陽性妊婦の多くが経済的に余裕のないマイノリティグループに属しており，合併症が多いという社会的・医学的事情によるものと推測されるが，わが国の国情には合致しないと思われる。母乳を介した HIV 母子感染は明白な事実である。表 3 に研究協力者，熊曙康の中国 (ROC) における成績を示したが，人工栄養に比して約 20% も母子感染率が上昇することが明らかである。HIV 陽性妊婦の授乳中の HIV viral load は不明であるが，わが国の現状では母乳哺育は避けるべきであろう。

当班では HIV 陽性妊婦の経膣分娩の可能性とその条件，更には母乳哺育実現のための基礎的検討を行っているが，わが国の現時点における国情を鑑みて，HAART，選択的帝王切開，新生児への生後 6 週までの AZT シロップ投与，及び人工栄養の組み合わせは当分の間 HIV 母子感染対策のゴールデンスタンダードである。

文　献

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Plasma levels of α-defensins 1–3 are an indicator of neutrophil activation in pregnant and post-partum women

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Abstract

Aim: In severe preeclampsia and septic shock, excessively activated neutrophils are thought to injure tissue irreversibly. On the other hand, mild neutrophil activation is known to occur during normal pregnancy. The objective of this study was to determine whether elevated plasma levels of α-defensins 1–3 could be used as an indicator of neutrophil activation in pregnant and post-partum women.

Methods: Defensin concentrations in 21 non-pregnant women and men, 184 normal pregnant women, and 55 post-partum women were quantified using an enzyme-linked immunosorbent assay (ELISA). The expression of the surface markers, CD11b and Toll-like receptor-4 (TLR-4), on the neutrophils were analyzed by flow cytometry in a cohort of subjects different from that used for the analysis of α-defensin levels.

Results: The concentrations of α-defensins were significantly higher in women that were in labor than in any of the other subjects. These levels diminished after delivery, but remained significantly elevated at one month post-partum. The expression of both CD11b and TLR-4 was significantly higher in women in labor compared to non-pregnant donors (controls). CD11b expression remained high on the third post-partum day, while TLR-4 expression fell to non-pregnant levels.

Conclusion: Our results suggest that there is a positive association between defensin levels and neutrophil activation in pregnant and post-partum women.

Key words: CD11b, defensin, labor, neutrophil, TLR-4.

Introduction

Neutrophils play an important role in the innate immune response to infection in mammals. These cells produce antimicrobial α-defensins 1–3 that are stored in specific, azurophilic granules and are released in response to infection.[1] Stimuli such as infection and injury induce the activation of neutrophils, which respond through phagocytosis and the release of antimicrobial peptides such as α-defensins 1–3. In addition to further activating the neutrophils themselves, these defensin molecules promote migration and phagocytosis by other phagocytic cells such as macrophages, and stimulate mast cells to release histamine, which increases vascular permeability and promotes the accumulation of more inflammatory cells. Finally, defensins, as well as other antimicrobial peptides increase the production of endothelial interleukin-8, which further promotes inflammation.[2]

Mild activation of neutrophils has also been noted during normal pregnancy in response to syncytiotrophoblastic, apoptotic debris that enters the maternal circulation.[3] Once labor begins, the amount of tissue debris that enters the maternal circulation increases

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and induces a systemic inflammatory response.[4–8] The role played by neutrophils in this post-partum inflammatory process has not been investigated.

Preeclampsia, defined as high blood pressure and excessive protein levels in the urine after 20 weeks of pregnancy, is thought to be caused, in part, by the increased systemic activation of neutrophils,[9] poor placentation,[10] and, in more serious cases, maternal systemic inflammation.[11,12] In light of the reported association of activated neutrophils with this condition, an assessment of the degree of neutrophilic activation during pregnancy may be clinically useful in diagnosing preeclampsia in its early stages. In this study, we determined whether the measurement of plasma α-defensins 1–3 levels is useful in the assessment of the extent of neutrophil activation in normal pregnant and post-partum women.

Materials and Methods

The institutional ethical committee approved the entire protocol of this study.

Plasma concentration of α-defensins 1–3

Plasma samples were obtained from a total of 260 individuals after receiving their informed consent. The subjects included 21 healthy donors (the control group, 11 men and 10 non-pregnant women), 184 normal pregnant women ($n = 53$, 43, 52, 10 and 26 at 8–12 weeks gestation, 23–30 weeks gestation, 33–36 weeks gestation, 37–40 weeks gestation and not in labor, and 37–40 weeks gestation and in labor, respectively), and 55 women after term normal vaginal delivery ($n = 37$ and 18 within 1 week and at 1 month post delivery, respectively). Women 'in labor' were defined as women at the beginning of the active phase of first stage term labor. All of the pregnant women were outpatients at our clinic from June 2003 to January 2005. Exclusion criteria included a body temperature above 38°C, obvious symptoms of an infectious disease, and moderate to severe obstetric and systemic disorders, especially those accompanied by inflammation, such as diabetes, asthma, preeclampsia, or threatened premature delivery.

All venous blood samples were harvested under uniform conditions into heparinized tubes that were immediately centrifuged at $300 \times g$ for 30 min to separate out the plasma and to prevent the degranulation of neutrophils that would have occurred during blood clotting.[13] The concentration of defensins in these samples was assessed using an immunoassay as previously described.[14] Specifically, the plasma concentrations of α-defensins 1–3 were determined using a sandwich enzyme-linked immunosorbent assay (ELISA) kit (Human HNP 1–3 ELISA Test Kit, HyCult Biotechnology b.v, Uden, the Netherlands) following the manufacturer's instructions. Each sample was diluted 1000-fold and each assay was run in duplicate in a microplate, which was read in a microplate reader (Biotrak II Visible Plate Reader, Amersham Biosciences Corp., Piscataway, NJ) at an optical density of 450 nm.

Neutrophil counts

Neutrophils were counted as part of routine check-ups that the 136 women underwent during and after their normal pregnancy. The blood samples used to obtain plasma were also used to quantify α-defensins 1–3 levels. Neutrophil counts were calculated by multiplying the total number of leukocytes by the percentage of neutrophils. The leukocyte counts and neutrophil fractions were obtained using an automated leukocyte differential system (CELL-DYN 4000, ABBOT JAPAN, Tokyo, Japan). The normal ranges for leukocyte counts and the neutrophilic fraction in our hospital have been set to $4.0–9.0 \times 10^9$/L and 37–73%, respectively.

Analysis of neutrophil surface markers

Venous blood was harvested from seven non-pregnant healthy women, seven healthy pregnant women in labor, and seven women three days after normal delivery. These subjects were different from those included in the analyses above. Neutrophils were isolated within 90 min of harvesting using a density gradient method with 2% dextran and Ficoll-Paque solution (Ficoll-Paque PLUS, density = 1.077, Amersham Biosciences Corp.). Expression of both CD11b and Toll-like receptor-4 (TLR-4) on the surface of neutrophils was determined using flow cytometry (FACSCalibur, Becton Dickinson, Franklin Lakes, NJ). A cell suspension of 5×10^5 cells in 500 μL of 0.1 M phosphate buffer solution (pH 7.2) was treated with 10 μL PE-antihuman monoclonal CD11b antibody (eBioscience Inc., San Diego, CA) at 4°C for 20 min or with 1 μL PE-antihuman monoclonal TLR-4 antibody (eBioscience Inc.) at room temperature for 15 min. PE-antimouse IgG2a monoclonal antibody (eBioscience Inc.) was used as the control antibody. The cells were washed and resuspended in 2 mL phosphate-buffered solution, then analyzed by flow cytometry. Neutrophil purity, assessed by size and granularity during flow cytometry, was greater

Table 1 Characteristics of this study and neutrophil counts

Groups	*n*	Age (y-o)	Neutrophil counts (10^9/L)
Healthy nonpregnant individuals		29.5 ± 3.9*	3.83 ± 1.30
Men	11		
Women	10		
Normal pregnancy			
8–12 weeks (1st trimester)	53	29.6 ± 5.2*	5.62 ± 1.56
23–30 weeks (2nd trimester)	43	29.7 ± 4.6*	6.43 ± 1.39
33–40 weeks (3rd trimester)	62	30.6 ± 5.1*	5.84 ± 1.93
37–40 weeks (in trimester)	26	32.1 ± 5.3*	8.71 ± 1.35**
Normal puerperium			
<1 week	37	30.8 ± 6.2*	6.98 ± 2.17
1 month	18	29.0 ± 5.9*	4.21 ± 0.76

*$P = 0.57$ (one-way ANOVA); **$P < 0.05$ versus all other groups (Scheffe's F test); Data is presented as the mean ± standard deviation.

than 91%. Twenty thousand cells were analyzed in each assay using CellQuest software (Becton Dickinson).

Statistical analyses

After performing a Bartlett test for homogeneity of variance, one-way ANOVAs were used to evaluate whether there were any age differences among the groups. The significance of differences in the concentrations of α-defensins 1–3, neutrophil counts and the expression of neutrophil surface markers among groups was determined using a Kruskal–Wallis test for non-parametric analysis of variance followed by Scheffe's *F*-test. $P < 0.05$ was considered statistically significant.

Results

Plasma concentrations of α-defensins 1–3

There were no significant differences in the mean age of our 260 subjects among the groups (Table 1). The plasma α-defensin 1–3 concentrations for each of our experimental groups are illustrated graphically in Figure 1. There was no significant difference in the median defensin concentrations between non-pregnant healthy women and healthy men (median ± SEM = 45.0 ± 39.0 vs 35.8 ± 26.3 ng/mL, respectively). The median value for all non-pregnant individuals was 39.0 ± 8.1 ng/mL, while the median concentrations for pregnant women at 8–12 weeks gestation, 23–30 weeks gestation, 33–36 weeks gestation, 37–40 weeks gestation and not in labor, and 37–40 weeks gestation and in labor were 38.9 ± 14.3 ng/mL, 71.8 ± 21.0 ng/mL, 80.0 ± 17.5 ng/mL, 96.8 ± 21.1 ng/mL, and 275 ± 85.6 ng/mL, respectively. The concentrations of α-defensins 1–3 in the plasma of women who had delivered their baby

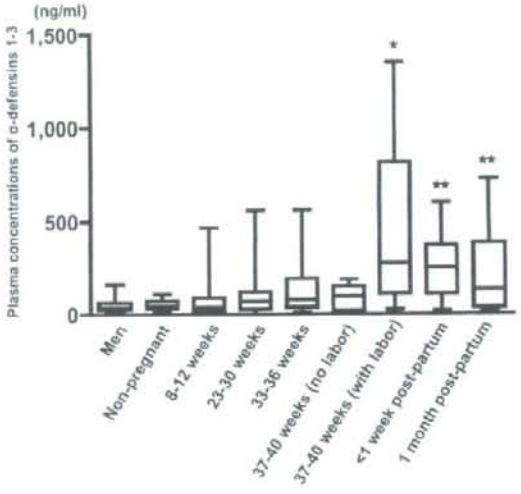

Figure 1 Median concentration and dispersion of plasma concentrations of α-defensins 1–3 during normal pregnancy and post-partum. Blood samples were obtained from non-pregnant healthy donors, normal pregnant women (8–12 weeks gestation, 23–30 weeks gestation, 33–36 weeks gestation, 37–40 weeks gestation and not in labor, and 37–40 weeks gestation and in labor), and women after normal term vaginal delivery (within 1 week and at 1 month post delivery). The error bars indicate the standard error of mean. *$P < 0.001$ versus all other groups, **$P < 0.05$ versus control (Scheffe's *F*-test).

within 1 week and 1 month earlier were 251 ± 34.2 ng/mL and 134 ± 60.8 ng/mL, respectively. The median plasma defensin concentration was significantly higher for women in labor than for any other group, and levels remained significantly higher than in the control group at 1 month post-partum.

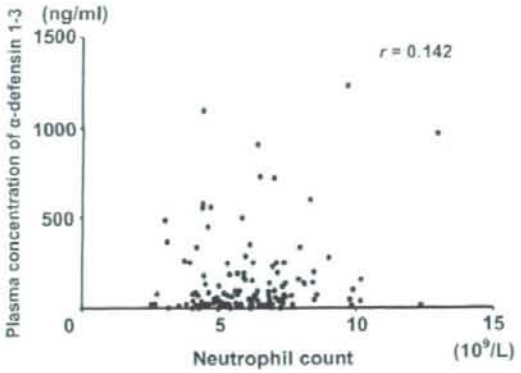

Figure 2 Scatter plot of the concentration of plasma α-defensins 1–3 and neutrophil counts during pregnancy and post-partum. Neutrophils were counted as part of routine check-ups that the 136 women underwent during and after their normal pregnancy using the same blood samples from which plasma was obtained to quantify their α-defensins 1–3 levels. $p = 0.105$ (Spearman's correlation coefficient by rank test).

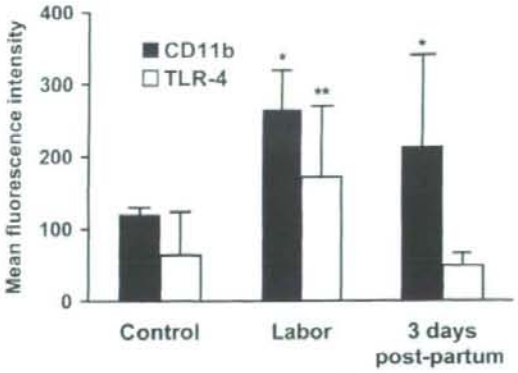

Figure 3 Quantification of surface marker expression on neutrophils. The solid bar indicates CD11b and the open bar indicates Toll-like receptor-4 (TLR-4). Blood samples were obtained from non-pregnant healthy women (controls), normal pregnant women in labor, and women at 3 days post-partum. The error bars indicate standard deviation. $^{*}P < 0.05$ versus control, $^{**}P < 0.05$ versus all of the other groups (Scheffe's *F*-test).

Correlation between α-defensins 1–3 plasma concentration and neutrophil counts

The same samples that were used to quantify defensin levels were used to count the number of venous blood neutrophils. Our results showed that women in labor had significantly higher neutrophil counts than subjects in any of the other groups (Table 1). After delivery, neutrophil counts fell quickly, reaching non-pregnant values by one month post-partum. There was no correlation between defensin concentrations and neutrophil counts (Fig. 2).

Expression of TLR-4 and CD11b on neutrophils

The mean fluorescence intensity of TLR-4 expression was 64 in the control group, but 172 in women in labor ($P < 0.05$). On the third post-partum day, TLR-4 expression fell to 49, which was not significantly different from controls. The mean fluorescence intensity of CD11b expression was significantly elevated in women in labor (266) compared to that in controls (120; $P < 0.05$). On the third day post-partum, CD11b expression remained significantly elevated (214) (Fig. 3).

Discussion

We have found no published reports detailing the levels of α-defensin during and after pregnancy. Evidence suggests that the number of neutrophils increases steadily during pregnancy, decreases after delivery, and reaches non-pregnant levels by about 1 month post-partum.[9,14] Since α-defensins 1–3 are stored in neutrophilic granules and are only released by activated neutrophils, we hypothesized that the levels of these compounds in plasma may correlate with neutrophil activation rather than the number of neutrophils in peripheral blood. Elevations in the numbers of neutrophils during normal pregnancy are thought to be due to delayed apoptosis.[15] TLR-4 was reported to be the principal regulator of neutrophil survival, with TLR-4-mediated neutrophil survival dependent upon signaling via the NFκB and mitogen-activated protein kinase cascades. TLR-4-activated monocytes were also found to play an essential role in delaying neutrophil apoptosis by releasing neutrophil survival factors.[16]

Although neutrophil CD11b expression has been reported to be increased in normal pregnant women[17] and the plasma level of elastase, granular components derived from azurophilic granules of neutrophils, is elevated in normal pregnancy,[18] there were no significant differences observed in the plasma α-defensin 1–3 concentrations between normal pregnant groups without labor and the non-pregnant group. The reasons why the plasma α-defensin 1–3 concentrations

were different from previous reports of CD11b expression and elastase in non-pregnant and normal pregnancy without labor could not be determined because the correlations between defensins and CD11b expression or elastase concentration were not analyzed in this study. The large dispersion of the plasma α-defensin 1–3 concentrations in normal pregnant groups may explain the inconsistency with previous reports.

Our data failed to reveal a correlation between plasma α-defensin levels and neutrophil counts during pregnancy and labor. We hypothesized that plasma α-defensin levels may reflect neutrophil activation, but not overall numbers. We evaluated the intensity of expression of CD11b, an established marker of neutrophil activation,[19–21] and TLR-4, a marker of delayed apoptosis in neutrophils, at the onset of labor and on the third post-partum day. CD11b expression and plasma α-defensin levels were significantly elevated in pregnant women in labor and in women in the early post-partum days compared to controls. TLR-4 expression was also significantly elevated during labor, but fell to non-pregnant levels by the third post-partum day. These results suggest that plasma α-defensin levels correlate with neutrophil activation rather than delayed apoptosis, i.e. the number of neutrophils.

Systemic inflammatory response syndrome (SIRS) is a critical condition that is often observed in patients in the intensive care unit who are suffering from septic shock, serious burn or other types of injury, or acute pancreatitis.[22] Both SIRS and severe preeclampsia are characterized by several clinical signs that include acute renal failure, intravascular disseminated coagulation (DIC), acute respiratory distress, and over-activation of neutrophils.[15] Neutrophils are also thought to help trigger labor, which in this context can be thought of as resulting from an inflammatory process, especially since they seem to accumulate in the lower rather than upper segment of the myometrium.[4] Thus, a large amount of α-defensin 1–3 would probably be released in severe preeclampsia and contribute to the cervical ripening that occurs during spontaneous labor and in cases of threatened premature delivery.

In conclusion, our results suggest that there is a positive association between defensin levels and neutrophil activation in pregnant and post-partum women. Further work is required to determine the significance of this data in predicting clinical outcomes in patients with preeclampsia or threatened premature delivery. It will contribute to resolve these complications during pregnancy.

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総　説

HIV 感染夫婦の生殖補助医療

Assisted Reproduction Techniques for HIV Positive Couples

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AIDS が死の病であった時，HIV 感染者が子供を希望しても子供を作らないように指導するのが医療従事者の職務とされていた。しかし 1996 年にプロテアーゼ阻害剤（PI）を含む多剤併用療法が導入された後 AIDS 死亡者は激減し，HIV/AIDS はコントロール可能な慢性感染症になりつつある。そのため，HIV 感染者の延命が可能となり，感染者は QOL の改善を求めると共に，結婚して子供が欲しいと願う夫婦が増えている。しかし，主治医に子供の相談をしても危険性を指摘されるだけで打開策がなければ，子供をあきらめるか，危険を覚悟して性交渉で子供を作るしかなく，結果として妻が感染した場合もあった。また，HIV に感染した児の予後は未だに不良で，抗 HIV 剤の催奇形性の問題もある。著者らは母も子も感染させないために，HIV 感染男性の精液から HIV を完全に除去し，HIV 陰性女性との間で 100% 安全に子供ができる方法を開発した[1]。HIV 感染症の治療の進歩と共に HIV 感染者の生殖補助医療も進歩しており，医療従事者は各方法の特徴や安全性を理解して感染者夫婦に説明する必要がある。

HIV 陽性夫婦の生殖医療を考える場合，以下の組み合わせがある。

1. 夫が HIV 陽性で妻が HIV 陰性の場合
2. 夫も妻も HIV 陽性の場合
3. 夫が HIV 陰性で妻が HIV 陽性の場合

これらについて，現状での対応を検討した。

夫が HIV 陽性で妻が HIV 陰性の場合

まず，HIV 感染男性の精液の特徴について述べる。

HIV 感染者の精液所見（図 1）

精液中には精子の他に精漿に浮遊する HIV RNA や精液中の単核球 HIV DNA から作られる HIV RNA などが含まれる。HIV 陽性男性では精子数の減少，精子の運動率低下，精子の奇形率増加が高率に認められる。

精液中 HIV RNA 量と血中 HIV RNA 量との相関（図 2）

我々の検討では精液中 HIV RNA 量と血中 HIV RNA 量には正の相関を認めた[2]。すなわち，血中ウィルス量が多いと精液中のウィルス量も多く，2 次感染の危険性も高い。逆に，治療で血中 HIV RNA 量が検出限界以下になると精液中の HIV RNA 量も低くなり，2 次感染の危険性も低くなる。しかし，精液中の白血球 HIV DNA がなくなることはなく，感染の危険性があると報告されている[3]。

性交渉による 2 次感染の危険性

HIV 感染男性が排卵日だけコンドームを使用しないで妊娠を図る場合でも毎年約 4.8% 前後の妻が感染すると推測される[4]。

人工授精による 2 次感染の危険性

HIV 陽性者の無処理の精液を用いた人工授精では約 3.5% が 2 次感染した[5]。それに対して，Semprini らは，Swim up 法により精子を処理して現在までに 2000 回以上人工授精を行い，2 次感染がないと報告している[6]。しかし，Semprini らの方法はウィルス除去率が十分でなく，HIV DNA の測定も行っておらず，100% 安全とは言えない。最近は，一般的には図 3 のように Pureception などを用いて精液を遠心処理し，Swim up 法で精子を回収し，人工授精や体外受精を行うことが多い。

ウィルス除去を目的とした Swim up 法の開発（図 4）

Swim up 法といっても施設によって方法が大きく異なり，結果としてウィルス除去率も異なる。一般的に行われている Swim up 法（図 3＋図 5 左）は不妊症で使用されてきた方法で運動精子の回収を目的としており，HIV 除去が十分とはいえない。

精液には前立腺由来の成分の他に，尿と同様に白血球や金属結晶，尿道通過時の雑菌や下着の繊維などが混入している。図 4 に示すように，著者らは遠心前に精液を希釈して静置し，精子より重い成分を沈殿させた後に上層の精子

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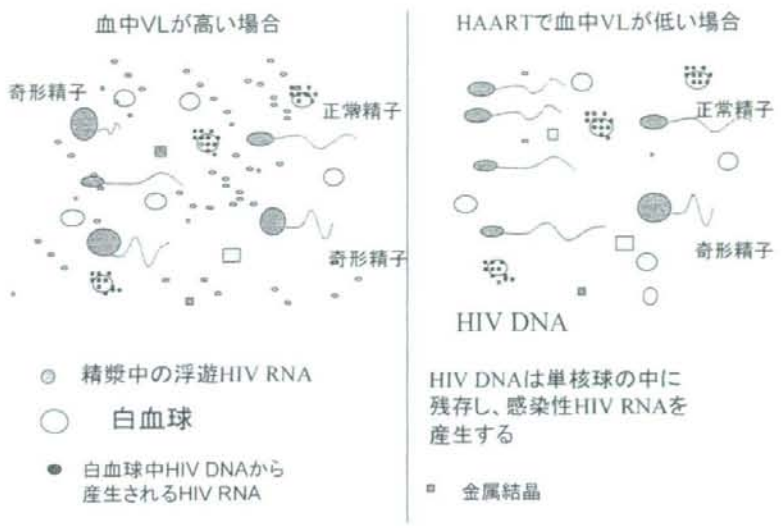

図 1

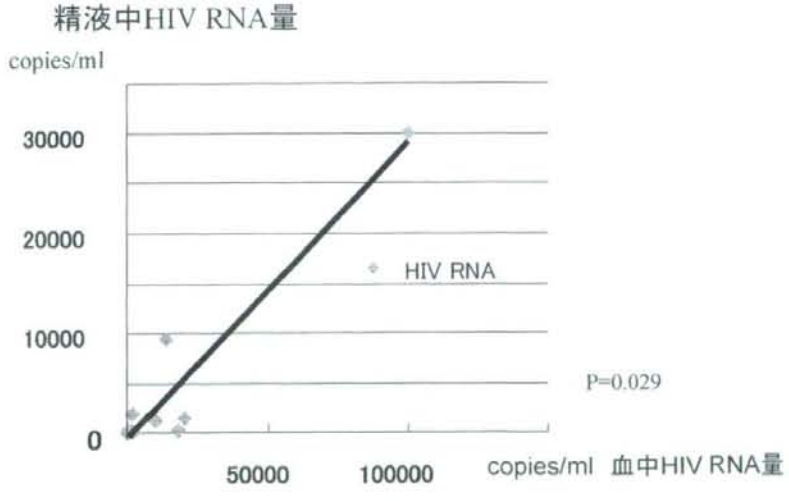

図 2

浮遊液を回収し，フィルター濾過して遠心分離した。

使用する分離液や濃度設定によってもウィルス除去率が異なる。Semprini らは当初 1 層のみのリンパ球分離液を用いた成績を発表したが，最近は一般に 90% と 45% の 2 層の Pureception が使用されることが多い。パコールにはエンドトキシン残存問題があり Pureception が用いられているが，Pureception による HIV 除去効率はパコールよりも低い[7]。著者らは 98% パコールを用いた連続密度勾配により HIV 除去率を $1/10^6$ 以下に高めた[1]。しかし，ウィルス除去率を高めると精子の回収率が悪くなる問題がある。

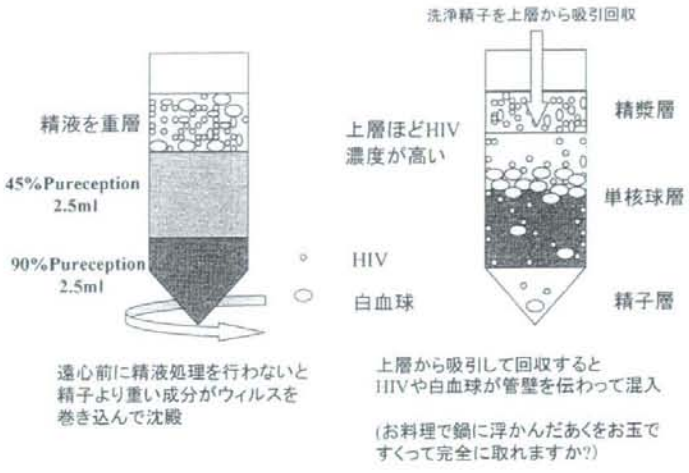

図 3

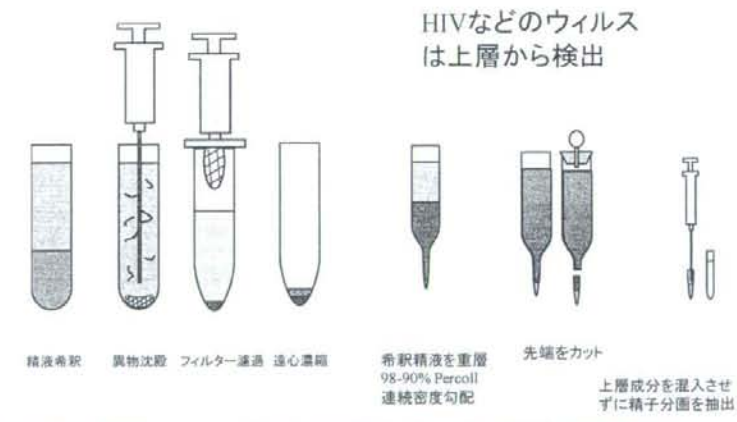

図 4

遠心分離後に，一番底の洗浄精子をどのように回収するかも問題である。遠心分離後，HIV は上層に分布し，単核球は中間層に分布している。図 3 に示すような従来法では，上から吸引して最下層の精子を回収しており，管壁を伝わり上層の HIV や白血球が必ず混入する。著者らは図 4 のように兼子が開発した特殊試験管を用いて上層成分を完全に遮断して底の精子を回収した。

従来の Swim up 法（図 5 左）は精子浮遊液に培養液を重層して泳いで上る精子を回収する。遠心後の精子を培養液で洗浄し，そこに培養液を重層すると比重差が少なくウィルスや白血球を攪拌してしまう。著者らは図 5 右に示すように 98% パコール液の最下層の精子液を底に乱流が生じ

図 5

ないように静置し，泳いで上る精子を回収している。

精子や卵への HIV の感染と付着の可能性

HIV が精子や卵に付着したり，感染するか否かも長く議論されてきた。精子や卵は原始細胞で，CD4 は発現していない。HIV が精子表面の糖脂質に付着するとの報告もあったが[8]，我々の方法で回収した精子には HIV は全く検出されず，付着もしていないことが確認された[1]。卵表面には CD4 蛋白はなく，体外受精の培養液にウィルスが混入しても，卵にウィルスが感染することはない[9]。しかし，HIV の混入した培養液を子宮内に戻すと感染の危険があるので完全除去が必要である。

体外受精と人工授精の比較：人工授精の抱えている危険性と課題

現在世界中で行われている人工授精では，実際に子宮に注入する精子のウィルス除去を確認しておらず，極めて大きな問題を抱えている。HIV の除去を PCR で確認するためには長時間を要し，その間に精子が損傷して受精率が低下するため実際には検査が行われていない。事前に同様の方法で HIV 除去を検査しているが，その場合ですら検出限界以下にできている確率が低く，不安を訴えて海外から相談に来た夫婦もいる。我々は HIV 除去の確認を 4〜6 時間以内に行える超高感度 PCR の開発に成功しており，今後，その間も精子機能を維持する方法を開発し，安全な人工授精が実施できるように検討している。

体外受精で卵を取り出して精子と培養する時に HIV がもし混入していたとしても卵には感染せず，HIV の感染性は 2 日目には 10 分の 1 以下に低下する[1]。さらに培養液を 2 日目に交換し，受精卵を洗浄すると，たとえ精子液に HIV が僅かに混入していても相当減らせる。我々は胚移植前に培養液中の HIV が全くないことを超高感度 PCR で確認している。そのため体外受精は人工授精に比して 2 次感染防止の面では数段優れている。

体外受精の課題

体外受精にはいくつかの課題がある。まず，医療費に保険が適応されず，排卵誘発剤などの費用が高い。また，排卵誘発剤投与による女性の負担が大きく，卵巣過剰刺激症候群などを合併すると腹水の治療が必要な場合もある。排卵誘発剤の注射などで妻には 2 週間前後の通院が必要となる。夫の HIV 感染について周囲に話していない場合が多く，妻の家族や職場などに事情を説明しにくいので，対応を相談する必要がある。

顕微授精（ICSI）について（図 6）

体外受精でも受精しないほど回収精子数が少ない場合もあり，図 6 のように運動性の良い精子を顕微鏡下で回収して卵細胞に注入する ICSI が必要となることも多い。注入精子液に HIV が混入している可能性があるので，事前の精子処理は必要不可欠である。ICSI 自身の安全性を問題視する意見もあり，ICSI により生まれた子供の異常率なども今後検討していきたい。精子の運動機能が良好なことと精子の DNA 損傷が少ないことには関連がないことも判明している。我々は，今までのような運動精子のみを選択する方法ではなく，兼子が開発した DNA 損傷がない精子を選択する技術を検討している。

実際の実施方法

研究班への挙児希望者は荻窪病院を受診して説明を受け，夫婦個別カウンセリングで参加意思確認後，精液のス

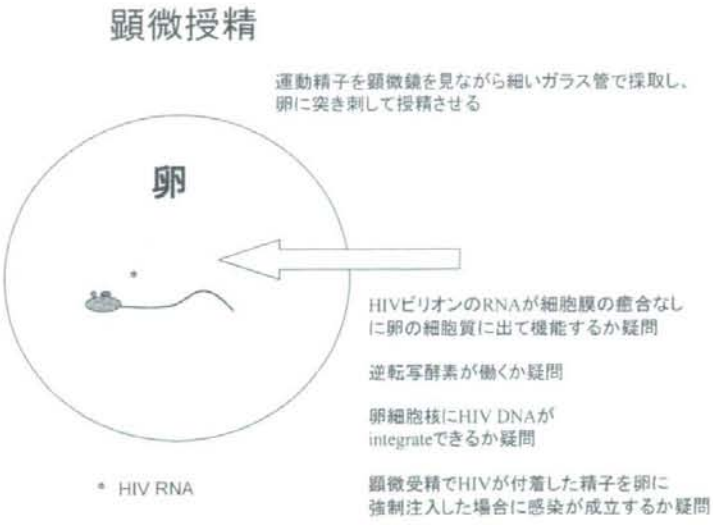

図 6

クリーニング検査を行う。その後，産科施設に紹介して改めて説明を受け，同意書を得た後に実施となる。胚移植後は妊娠の有無に係わらず妻の感染症検査を行い，出産後は母子の検査で2次感染がないことを確認する。

HIV 陽性男性と HIV 陽性女性の場合

HIV 感染者同士が結婚した場合の挙児相談や，夫婦間で感染した場合の相談も寄せられている。今後，HIV 感染者の予後が改善すると共に感染者同士の結婚も増え，挙児相談も多くなると推測される。この場合にどのような対応をとるのかについて，現状では倫理的にも社会的にも幅広い議論が必要である。その妥当性を判断する前提として，superinfection，HIV の変異，薬剤耐性，HIV 感染者の精子機能，HAART の精子への影響など多くの知識が必要であり，感染者夫婦個々の状況に応じた対応が求められる。

Superinfection（図 7）防止目的

HIV は変異速度が速く，宿主の免疫状態によっても感染者個々の HIV diversity は大きく異なる。既に HIV に感染していても別の HIV に再度感染すると，図 7 に示すように，HIV の再構築（recombination）が生じ，AIDS 発症を早めたり薬剤耐性を誘導する危険性がある[10]。また，たとえ夫婦間で感染したとしても夫婦の major clone が異なっていたり，時間と共に大きく変異し，性交渉で再感染が生じる可能性がある。最近，薬物注射による新規 HIV 感染者では約 5% に superinfection が生じているとの報告もあるが[11]，理論上はもっと高い可能性がある。superinfection を防止するためには，HIV 陽性同士でも生殖補助医療が必要となる。

耐性ウィルスの感染防止目的

夫婦のどちらかのウィルスが薬剤耐性となっていると，性交渉で新たに耐性ウィルスに感染する危険性がある。そのために生殖補助医療による予防が必要となる。

夫の精子機能が不良で男性不妊の場合

HIV 感染男性は治療の有無にかかわらず精子機能が傷害されている場合が多い[1]。また，我々は，HAART によって精子のミトコンドリア障害が生じ，精子の運動機能が障害され，男性不妊となっている場合があることを報告した[12]。HIV 感染男性では男性不妊となっている場合も多いと推測される。夫の精子機能が低下している場合は生殖補助医療が必要となる。

症例検討

夫婦共に HIV 陽性といっても多様な状況があり，実際に経験した case の一部を紹介する。

Case 1

夫婦共に凝固因子製剤によって HIV に感染したが，感染から 20 年以上経過しても無治療で免疫が良好に保たれている長期未発症者同士が最近結婚して子供の相談に来た。妻は長年血中 VL＜50 を保ち免疫も良好であるが，夫は最近 VL が 5 万を越えている。性交渉では妻に夫の増殖力の強い HIV が感染して妻の免疫を悪化させる危険性がある。夫が HAART を開始しても結局精液中の HIV DNA は消えず，感染の危険性は残るし，副作用などを総合的に考え，夫婦は HIV 除去精子による体外受精を希望している。

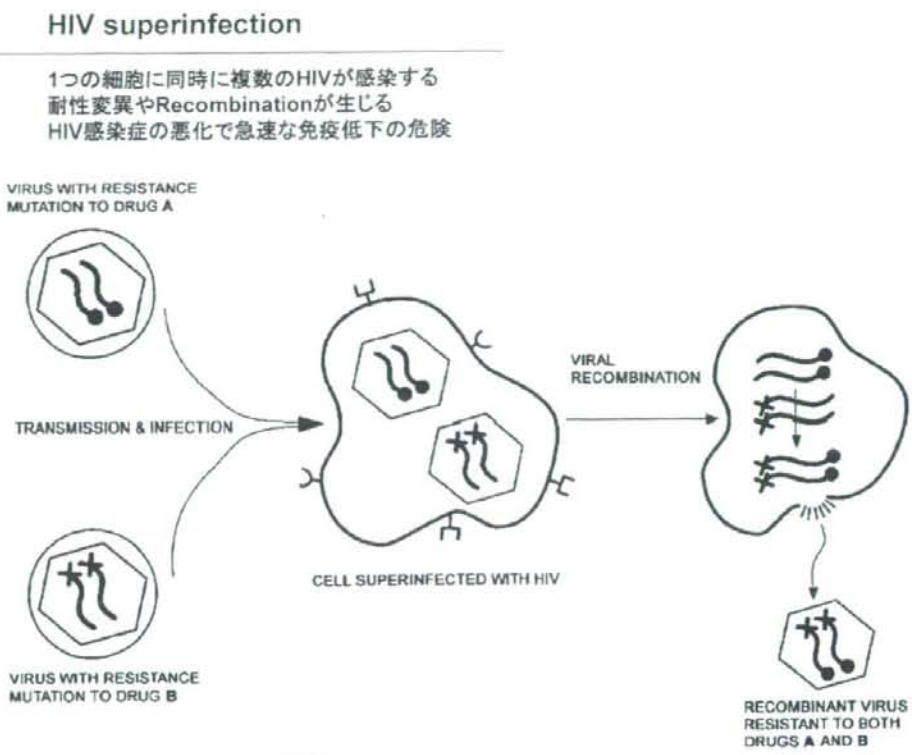

図 7

Case 2

高度の薬剤耐性となっている夫から妻に HIV 感染したが，妻の HIV は薬剤感受性があり治療でウィルスを抑制できている。性交渉で何年も子供ができないため相談に来たが，夫の精子機能障害を認めた。このまま性交渉を続けると薬剤耐性ウィルスが妻に感染する危険性があるので，HIV 除去精子による体外受精を希望している。

Case 3

夫から妻に感染したが，その後夫は治療によって HIV VL＜50 となり，子供の相談に来た。妻のウィルスは薬剤感受性が保たれており，夫の薬剤は有効であった。双方への superinfection の危険性を考えた場合，夫の精子機能が保たれていれば性交渉での妊娠を目指すか，人工授精などを行うかは夫婦とよく相談して決める必要がある。

このように夫婦共に HIV 陽性といっても状況によって対応を検討する必要がある。

倫理的課題

HIV 感染者の生殖補助医療に関して，夫婦共に HIV 陽性の場合は子供を持つべきではないと 2004 年に EU から勧告されている[3]。しかし，その根拠は，夫婦共に HIV に感染していると子供が成人するまでに両親共に死亡する可能性が高く，孤児になると不幸だからと記載されている。この勧告においては，長期未発症患者や superinfection については全く想定されていない。また，HIV 感染症の治療が進歩して予後が改善された場合は見直しが必要としており，夫婦ごとの状況に応じて検討すべきと考えられる。夫婦が共に病気を持っている場合に子供を持つ場合の是非論は HIV/AIDS に限ったことではない。ただ，HIV/AIDS の最新の医療情報をしっかり得た上で議論すべきである。

HIV 陰性男性と HIV 陽性女性の場合

基本的に夫から精液を採取して人工授精を行えば夫に感染することなく，子供を持てるので，妊娠後の母子感染を防止すれば大きな問題はない。ただ，我々は外国人夫婦で男性が精管結紮切除術を行っており，やむなく夫の精巣内精子採取術（Testicular sperm extraction : TESE）を行い体

外受精で妊娠したケースを経験している。

母子感染予防対策

HIV 感染女性が妊娠した場合，母子感染を防ぐための対策が必要である。母親の血中 VL をできるだけ低く抑制する HAART により，胎内感染を防止し，出産時の感染リスクを低くすることが証明されている。さらに陣痛が来る前の選択的帝王切開や，母乳禁止，新生児への抗 HIV 剤の投与などを組み合わせることにより，現在では HIV の母子感染率を 2% 以下に抑制できると報告されている[14]。しかし，耐性ウィルスへの対応方法や母体のウィルス量（RNA，DNA）をどれだけ抑制すれば帝王切開や新生児への抗 HIV 治療の必要性がなくなるのかなど今後検討すべき課題は多い。また，新生児では ZDV の薬物動態の検討も血中濃度測定も殆どされておらず，HAART の検討も十分されていない。ZDV 単独投与後の突然死も報告されており，新生児への投与量や投与期間が妥当かどうか，新生児の腎機能などによる調整の検討も必要と思われる。

成人に比べて小児科領域での新薬の開発は遅く，小児 HIV 感染例の予後もよくない。現状では母子感染を最大限に防止することが必要である。

C 型肝炎の問題

非加熱製剤を使用した血友病患者の 90% 以上が C 型肝炎にも感染し，HIV/HCV 感染者の死因の多くが C 型肝炎の悪化となっている。C 型肝炎の治療として PEG IFN＋ribavirin 併用療法が有効で，早期治療が推奨されている。リバビリンは精子の奇形を高率にもたらし，治療中及び治療終了後半年は避妊が必要とされている。しかし，我々の検討では治療終了後 1 年以上経過しても精子の異常が続く場合や，精子数の減少を認めた場合もある。C 型肝炎の治療を優先させるか，挙児を優先させるかは主治医とよく相談し，場合によっては精子の冷凍保存の必要がある。Swim up 法で精液中の HCV も除去される一方，体外受精で HCV が 2 次感染した報告もあり，生殖補助医療においてもウィルス感染対策が必要である[15]。

最 後 に

EU では，HIV 感染男性と HIV 陰性女性の人工授精や体外受精に対し，2 次感染の危険性を少なくする手段として施行されているが，アメリカ CDC は未だに禁止勧告を解除していない。我が国では HIV 感染男性の精液処理と人工授精は各施設の倫理委員会の承認を得てから実施するように日本産婦人科学会が 2003 年に勧告を出した。夫婦の状況によって，人工授精，体外受精，顕微授精などの適用は異なる。医療従事者が最新情報を提示した上で患者夫婦とよく相談して決定すべきである。しかし，その前に HIV 感染者の生殖補助医療を実施できる医師や施設を増やすことが急務である。

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Feature Article

Factors determining prenatal HIV testing for prevention of mother to child transmission in Dar Es Salaam, Tanzania

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Abstract

Background: The objectives of the study were (i) to evaluate the Prevention of Mother to Child Transmission (PMTCT) services in Temeke district, Tanzania and (ii) to identify factors for non-acceptance of HIV testing among pregnant mothers in the area.

Methods: A structured questionnaire was used in face-to-face interviews at five health centers in the district. Univariate and multiple logistic regression analyses were used to assess the association of the refusal of human immunodeficiency virus (HIV) testing with risk factors.

Results: Two hundred and seventy-three (68.1%) of the participants had already had HIV testing, while 128 (31.9%) had not. Participants' general knowledge of HIV was high, but specific knowledge of mother to child transmission (MTCT) was relatively low. In the multiple logistic regression analysis, frequencies of antenatal clinic visits, awareness of MTCT and intensive family support were significantly and inversely associated with the refusal of HIV testing.

Conclusions: Frequency of antenatal care visits, spreading information on HIV/acquired immune deficiency syndrome especially MTCT, and husbands' intensive support are significant factors for increase of HIV test acceptance among pregnant women in the study area.

Key words HIV testing, mother to child transmission, pregnant women, Preventing Mother to Child Transmission program, Tanzania.

Mother to child transmission (MTCT) of human immunodeficiency virus (HIV) is the major source of HIV infection in children. There are 40.3 million people infected with HIV globally and 2.3 million are children under 15 years of age. Sub-Saharan Africa remains hardest hit, with 25.8 million people living with HIV. Two-thirds of all people living with HIV are in Sub-Saharan Africa, as are 77% of all women with HIV.[1] In Tanzania, the first acquired immune deficiency syndrome (AIDS) cases were diagnosed in 1983.[2] During the 1990s the prevalence of HIV in Tanzania fluctuated greatly. In 2000 it was reported that approximately 1 800 000 people aged ≥15 were living with HIV. Of them, >900 000 women were of reproductive age (15–49 years).[3] The main mode of transmission is through heterosexual intercourse, accounting for 78% in 2001, and MTCT ranked second, contributing up to 5%.[2]

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In Tanzania, the estimated number of women who become pregnant per year is 1.3 million and HIV prevalence among women attending antenatal clinics in 2002 was 9.6%.[2,4,5] In addition, data from the Tanzania Ministry of Health (MOH) show that the risk of transmission from HIV-infected pregnant women to their newborns is 40%, distributed as follows: 10% *in utero*, 20% during labor and delivery, and 10% through breast-feeding.[4] The Preventing Mother to Child Transmission (PMTCT) of HIV program aims at reducing MTCT of HIV during pregnancy, childbirth and during breast-feeding. It includes voluntary counseling and testing (VCT), antiretroviral (ARV) treatment and counseling about feeding options (Fig. 1). In 1998, after finding that a short course of the antiretroviral drug Zidovudine (ZDV; GlaxoSmithKline, Middlesex, UK) starting from 36 weeks of pregnancy reduced the rate of MTCT by 50%, a comprehensive PMTCT strategy was developed. The PMTCT project in Tanzania was launched in 1999, and started operation from five centers. In 2002, an evaluation report for the project showed some weaknesses and constraints in PMTCT services.[6,7] One of the major barriers was its low

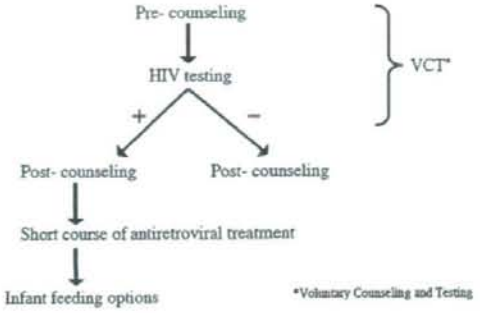

Fig. 1 Preventing Mother to Child Transmission.

acceptance. The report also showed large differences in counseling rates (9–56%), high acceptance of rapid HIV testing among those who received counseling (78–84%), and low short-course ZDV uptake (8–20%).

Temeke district was chosen for the following three reasons. First, the Dar Es Salaam region has the highest HIV/AIDS case rate (235 per 100 000) compared to other regions.[8] A previous survey showed that prevalence of pregnant women varies by geographical settings, with much higher levels in border towns, along major highways and in urban centers as compared to rural areas.[2] Second, in the Dar Es Salaam region, the Temeke district represents a typical urban city because the other districts are a center for government and commercial offices and a residential area for foreign and upper classes. Third, although coverage of PMTCT is very limited elsewhere in the country, the project had been extended to the health center level in Temeke.

No study has been done in Tanzania despite that acceptance of the program has been less than optimal. Therefore, the aim of the present study was to determine the reasons for low acceptance of PMTCT and to recommend proper measures to be taken in Tanzania. The objectives of the study were (i) to evaluate the PMTCT services that are currently provided and the knowledge and attitude on counseling and testing of HIV among pregnant mothers in Temeke district, Tanzania and (ii) to identify factors for non-acceptance of counseling and testing for HIV among pregnant mothers in the area.

Methods

Study area

The study was conducted at five health centers in Kasorobo, Mtoni, Rangi Tatu, Round Table and Tambukareli in Temeke district, Dar Es Salaam, Tanzania (Fig. 2). Tanzania has a total area of 940 000 km^2, and is divided into 26 administrative regions and 131 districts. The total population is estimated at

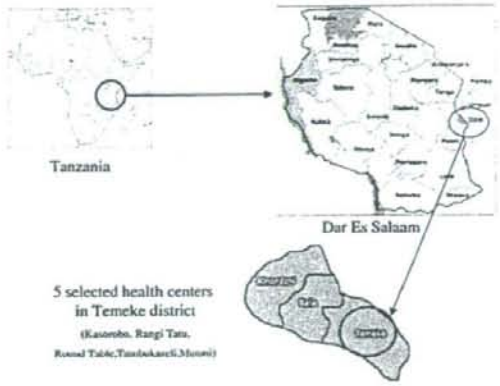

Fig. 2 Map of study site.

approximately 34.5 million and the population growth rate is 2.9% per year.[9] Although Dar Es Salaam is not a capital anymore, it is still the center of politics and economy. Temeke district (municipality) is in the southern part of the Dar Es Salaam Region. The region consists of three districts and Temeke is the largest one. It has an estimated population of 768 451 and the population growth rate is 7.8% (Temeke municipal council, unpubl. obs., 2004).

Study population

The target population consisted of pregnant women attending antenatal care at the selected health centers where PMTCT programs are implemented. Because the rates of access to antenatal care services were 98% (one visit) and 70% (four times or more) in Tanzania, random sampling was applicable in the present study.[10] Then the sample size was calculated from the previous records in the area using EpiInfo version 6 (Centers for Disease Control and Prevention, Atlanta, USA) and 401 pregnant women were enrolled.

Study design and procedure

This was an exploratory and cross-sectional study, conducted from August to September 2004. A structured questionnaire was used in face-to-face interviews with pregnant women at the health centers by a trained interviewer in the Swahili language. All women seeking antenatal care were approached individually and asked to participate in the study. Verbal consent was obtained from all participants. Each interview was conducted in an isolated space and lasted approximately 15–20 min. Incentives were not provided for participation. To preserve confidentiality and because of concerns about unnecessary disclosure, the participants' actual HIV status was not

collected. Permission was obtained from Tanzania Commission for Science and Technology, Ministry of Health in Tanzania and the Ethical Review Committee, Graduate School of Medicine and Faculty of Medicine, University of Tokyo.

Questionnaire

In order to identify factors associated with refusal of HIV testing among pregnant women, a structured questionnaire was designed. The dependent variable was 'not being tested for HIV'. Demographic factors (age, educational background, religion, marital status, family size and number of children), gestational age, frequencies of health center and antenatal visits, travel time from home to health centers, general knowledge of HIV, specific knowledge of MTCT of HIV, and attitude toward HIV testing were introduced as independent variables.[11]

For measuring attitude toward HIV testing, the modified HIV-Antibody Testing Attitude Scale developed by Boshamer and Bruce in 1999 was added to the questionnaire.[12] It was used to assess respondent concern about family support, social support, and privacy. Scores on the scales were positively correlated with perceived knowledge about HIV and the likelihood of being tested.[12]

Statistical analysis

Statistical analyses were performed using the SPSS 11.0J program (SPSS Japan, Tokyo, Japan). Univariate and multiple logistic regression analyses were used to assess the association of the refusal of HIV testing with each risk factor. The χ^2 test was used to compare the rates in different categories. Variables that had a significant relationship to the dependent variable at the $P < 0.05$ level were then used in the multiple logistic regression analysis and selected by using backward stepwise procedure.

Results

Characteristics of respondents and acceptance rate of HIV testing

Four hundred and one pregnant women were interviewed at five health centers in the Temeke district. The participants characteristics are given in Table 1. Two hundred and seventy-three (68.1%) had already had HIV testing, while 128 (31.9%) had not. The acceptance rate of testing in the present study was larger than that in 2003 (42.6%). Among the five health centers, no statistical difference was seen in those who had been tested and those who had not. None of the social demographic factors was significantly associated with an acceptance of the HIV testing.

Table 1 Participant characteristics ($n = 401$)

Variables	Mean	SD	*n*	%
Domicile				
Kasorobo			48	12.0
Mtoni			49	12.2
Rangi Tatu			120	30.0
Round Table			121	30.2
Tambukareli			63	15.7
Age (years)	24.18	5.53		
Gestational age (months)	6.84	1.85		
Education (years)				
≦7			362	90.3
>7			39	9.6
Marital status				
Married			323	80.5
Single			29	7.2
Others			49	12.1
Family size	4.34	2.51		
No. children	1.17	1.39		
Distance to health centers (min)	26.03	24.96		
Transportation				
On foot			290	72.6
By public bus			105	26.2
Others			6	1.5
Travel time (min)	30(mode)			
No. health center visits	2.01	1.34		
No. antenatal care visits	2.67	1.69		
HIV testing				
Tested			273	68.1
Not tested			128	31.9

Among those previously tested, 69.2% of participants ($n = 189$) answered that the final decision was made by themselves, and 52.3% ($n = 67$) of those who had not been tested responded that they would like to be tested in the future. Major reasons of refusing test were the following: 'I don't have enough information about testing' (58.4%), 'I don't know the benefits of knowing my HIV status' (40.4%), 'I can't get any medication if I am found HIV positive' (32.6%) and 'I'm afraid of knowing my HIV status' (31.5%). In contrast, the major reasons for acceptance of testing were the following: 'I'm interested in knowing the results' (98.2%), 'It's beneficial to me' (94.9%), 'It's beneficial to my baby' (94.9%), 'I had enough information about testing' (94.1%) and 'I'm not afraid of knowing the status' (93.4%).

Awareness of HIV

Participant awareness of HIV is shown in Figure 3. A total of 98.2% of those who had been tested for HIV and 98.4% of

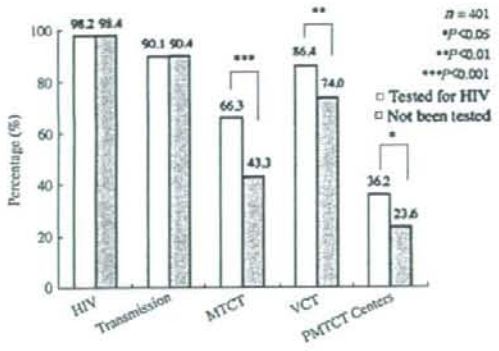

Fig. 3 Awareness of HIV. MTCT, mother to child transmission; PMTCT, prevention of mother to child transmission; VCT, voluntary counseling and testing.

those who had not been tested had heard of HIV/AIDS. A total of 90.1% of those who had been tested and 90.4% of those who had not understood how the disease is spread (main answers: sexual intercourse and sharing sharp edges). In contrast, the awareness of MTCT of HIV was relatively low: 66.3% of those who had been tested and 43.3% of those who had not, answered correctly. Sources of information about MTCT of HIV are given in Figure 4. Among those who were aware of MTCT of HIV, 68.4% answered that they learned about it from antenatal care visits, 43.7% from radio, and 26.9% from friends and neighbors.

Knowledge of MTCT of HIV

The knowledge of MTCT was also low, as shown on Table 2. The percentage of correct responses to the statement that HIV-positive pregnant women can transmit the virus to their babies during pregnancy was 49.4% of those who had been tested for HIV and 45.4% of those who had not been tested; for transmission during delivery it was 55.7% of those who had been tested and 50.0% of those who had not; and for during breast-feeding it was 63.5% of those who had been tested and 60.0% of those who had not, respectively. Moreover, 23.8% of those who had been tested and 20.3% of those who had not correctly answered that HIV-positive mothers always deliver HIV-positive babies. 38.7% of those who had been tested and 22.3% of those who had not knew of medicine for PMTCT of HIV, and statistical significance was found between these two groups.

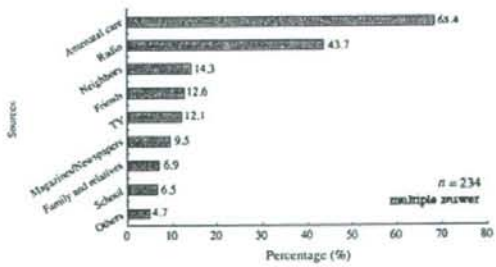

Fig. 4 Sources of information about mother to child transmission of HIV.

Table 2 Knowledge of MTCT of HIV

Queries	Percentage of correct answers	
	Tested for HIV (n = 273)	Not tested for HIV (n = 128)
MTCT during pregnancy?	49.4	45.4
MTCT during delivery?	55.7	50.0
MTCT during breast-feeding?	63.5	60.0
If a baby tests positive means a mother positive?	78.2	70.8
HIV-positive mother always deliver HIV baby?	23.8	20.3
Medicare for MTCT?	38.7	22.3***

***$P < 0.001$. MTCT, mother to child transmission.

Attitude toward HIV testing

The result of the attitude scale is shown in Table 3. It used a three-point Likert scale: agree, neutral, disagree. The mean score of family support (items: 'My family would support me if I decided to be tested for HIV', 'I could easily discuss HIV testing with my family' etc.) was 11.03 ± 3.204 (range: 9–27). On this scale, 9 was the strongest and 27 was the weakest evidence for family support. The mean score of the social support (items: 'I would not want anyone to know if I got an HIV test', 'People would assume I have HIV if I decided to get tested' etc.) was 12.15 ± 3.136 (range: 9–25). On this scale, 9 was the strongest and 25 was the weakest evidence for community support. The mean score of privacy (items: 'I am afraid that if I were to be tested for HIV, my name would go onto public records', 'HIV testing information is kept very confidential by the medical staff who do the testing' etc.) was 9.06 ± 2.414 (range: 7–21). On this scale, 7 was the strongest and 21 was the weakest evidence for protection of their privacy.

Table 3 Attitude scale toward HIV testing

	Range	Mean	SD
Family support	9–27	11.03	3.20
Social support	9–25	12.20	3.14
Privacy	7–21	9.06	2.41

Univariate analysis

Univariate analysis was used to assess the association of the refusal of HIV testing with potential risk factors. The result is shown in Table 4. Participants who were aware of MTCT of HIV, VCT and PMTCT centers, and the lower score of family support, social support and privacy were significantly inversely associated with refusal of HIV testing. Concerning knowledge of medication of PMTCT, the result did not reach statistical significance.

Multiple logistic regression analysis

Multiple logistic regression analysis was used to assess the association of non-acceptance of HIV testing with each risk factor. The following variables were used for selection of independent variables: (i) gestational age; (ii) number of health center visits; (iii) number of antenatal clinic visits; (iv) frequencies of antenatal clinic visit; (v) awareness of MTCT; (vi) awareness of VCT; (vii) awareness of PMTCT cites; (viii) knowledge of MTCT of HIV; (ix) family support; (x) social support; and (xi) privacy.

Table 4 Univariate analysis of HIV testing refusal

Variables	Crude OR	95%CI
No. antenatal clinic visits		
≥2	0.152	0.095–0.245
<2	1	
Awareness of MTCT of HIV		
No	2.575	1.671–3.968
Yes	1	
Awareness of VCT		
No	2.230	1.317–3.776
Yes	1	
Awareness of PMTCT centers		
No	1.834	1.136–2.962
Yes	1	
Knowledge of medication of PMTCT		
No/Don't know	1.132	1.004–1.277
Yes	1	
Score of family support		
>11	1.360	1.245–1.485
≦11	1	
Score of social support		
>12	1.156	1.081–1.236
≦12	1	
Score of privacy		
>9	1.277	1.166–1.399
≦9	1	

CI, confidence interval; MTCT, mother to child transmission; OR, odds ratio; PMTCT, prevention of mother to child transmission; VCT, voluntary counseling and testing.

The independent variables were selected using a stepwise procedure. Three independent variables (frequencies of antenatal clinic visits, intensive family support and awareness of MTCT) were used in the final model (Table 5). These were independently, significantly and inversely associated with refusal of HIV testing.

Discussion

Many pregnant women were willing to take the HIV test, but there were some constraints. Although previous studies noted that the demographic characteristics were associated with acceptance of HIV testing, none of that was statistically significant in the present study.[14–15] More frequent antenatal visits by the respondents was significantly inversely associated with non-acceptance of the testing in both univariate and multivariate analysis. Late or no prenatal care were variables independently associated with a lower probability of being HIV tested.[16] Beginning antenatal care early in pregnancy and frequency of health care and antenatal care visits might influence the increase of the test acceptance because the PMTCT program is carried out mainly by nurses or counselors.

As shown in the results, general knowledge of HIV was well-spread in the study area, but prevalence of specific knowledge of MTCT remained problematic. The results showed that the acceptance of HIV testing might increase when women understand MTCT of HIV and the role of medication, which is in agreement with previous studies.[14,17,18] Information and greater knowledge of MTCT of HIV are crucial in improving acceptance of the testing. The low levels of the knowledge can be explained by a lack of necessary information. Radio, friends and relatives as well as antenatal care were the main sources for knowledge of HIV/AIDS in the present study, which was consistent with previous reports.[2,19,20] Because the educational levels of respondents in the study were relatively low, the dissemination of information on HIV/AIDS may be effective

Table 5 Multiple logistic regression analysis of HIV testing refusal

Selected variables	Adjusted OR	95%CI
No. antenatal clinic visits		
≧2	0.571	0.450–0.724
<2	1	
Awareness of MTCT of HIV		
No	2.423	1.299–4.520
Yes	1	
Score of family support		
>11	1.358	1.231–1.498
≦11	1	

CI, confidence interval; MTCT, mother to child transmission; OR, odds ratio.