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Brief Report

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Physical activity and associated factors among young adults in Malaysia: An online exploratory survey

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Summary **The burden of non-communicable diseases is increasing in Malaysia. Insufficient Physical Activity, which is an important risk factor for non-communicable diseases, is less researched in Malaysia. We aimed to assess the level of physical activity and identify its correlates. An online survey was carried out during October, 2011 in the University Tunku Abdul Rahman by the opinion poll research committee. Young adults answered the Short International Physical Activity Questionnaire and a questionnaire about factors according to a socio-ecological model which was adapted from published studies. Metabolic equivalent (MET)-hours and MET-minutes were calculated. Physical activity was classified as sufficient when MET-minutes were > 840. The mean age of the 474 participants was 22.4 years (S.D. = 4.7), and 253 (53.4%) were females. Their mean and median of MET-hours of PA done during the previous seven days were 31.36 (S.D., 52.19) and 14.7 (IQR, 5.77-32.07), respectively. Physical activity done was sufficient among 242 (51.1%) participants. Using univariate analysis, being male, good self-rated health, positive intention, self-efficacy, perceived benefits, social support, and availability of facilities were associated with sufficient physical activity. Using multivariate analysis sufficient physical activity was associated with participants' intention (OR 0.75, 95% CIs 0.64, 0.88), self-efficacy (OR 0.91, 95% CIs 0.85, 0.97) and facility availability (OR 0.81, 95% CIs 0.73, 0.91). The proportion of participants with sufficient physical activity was low. Positive intention and self-efficacy associated with sufficient physical activity should be supported by availability of facilities and a safely-built environment. A nationwide survey about physical and associated social-ecological factors is needed to design rational health promotion strategies.**

Keywords: Physical Activity, metabolic equivalent, non-communicable diseases

1. Introduction

Physical inactivity is the fourth leading risk factor for global mortality which accounts for 6% of all deaths (*1*). Physical inactivity is an important risk factor for non-communicable diseases (NCDs) which is estimated to cause nearly 21-25% of breast and colon cancers, 27% of diabetes, and approximately 30% of ischemic heart disease (*2,3*). Recent data indicates that 60% of the world's population fails to meet the World Health Organization (WHO) recommendation that adults should participate in a minimum of 30 minutes of moderate or vigorous Physical Activity (PA) every day (*1*). Insufficient PA is associated with urbanization and technological advancement (*4*). Sustained economic growth and rapid urbanization may have resulted in a decreased level of PA among Malaysians. Moreover there is increasing concern about the growing burden of NCDs in Malaysia (*5,6*). A national survey about metabolic syndrome has reported that prevalence was

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30.1% according to the IDF (International Diabetes Federation) definition (*7*). Surveys about PA using self-reported questionnaires have reported that children aged 7-12 years have a preference for sedentary pursuits, rather than sports or active games during their leisure time (*8,9*). However, there is very little information about factors associated with PA among young adults in Malaysia. With rapid urbanization more young adults may be spending less time in PA due to increased use of motorized transportation rather than walking. Studies from developed countries have studied various patterns of PA and factors associated with PA (*10-13*). Such information may be useful for formulating strategies and testing interventions to improve PA level. There is a paucity of such studies in developing countries like Malaysia. The objectives of our survey were: to estimate the level of PA among young adults in a sub urban population and identify the factors associated with PA according to a social-ecological model.

2. Methods

2.1. *Design*

The current study was a cross-sectional online survey.

2.2. *Setting*

The survey was carried out by University Tunku Abdul Rahman (UTAR). The UTAR opinion poll survey is an initiative undertaken by a committee which has representatives from all faculties of the university. Each month, the committee plans and executes opinion poll surveys which are varied topics of general interest about youth in Malaysia (*http://poll.utar.edu.my*).

2.3. *Participants*

Young adults from the general population who had registered for UTAR opinion poll survey.

2.4. *Sampling and sample size*

No sampling method was adopted and sample size estimation was not done because this was an exploratory survey.

2.5. *Instrument*

The instrument for this survey was developed based on the framework used in previous studies reported from Canada and Malaysia (*8,12*). The survey used a social-ecological model to assess demographic, individual, social and physical environmental factors (*14*). The questionnaire and framework for our survey was adapted from a Canadian study with the author's permission (*12*). To measure physical activity, we used an English version of a short self-administered International Physical Activity Questionnaire (IPAQ) which is available at *http://www.ipaq.ki.se/scoring.pdf*. This version of IPAQ is meant for use among young and middle-aged adults and has an acceptable test-retest reliability and criterion validity (*15*). The participants were asked to report the number of days on which they did 1) vigorous PA, 2) moderate PA, and 3) walking during previous seven days from the day of survey. In addition, they were also asked to report number of hours and minutes they carried out these three types of activities on each day. In IPAQ, the questions about PA included activities they do at the work place, as part of household and yard work, getting from place to place, and during spare time for recreation, exercise or sports. However, no information was asked about frequency and duration for these separate domains of PA. In addition to IPAQ, we collected information about socio-demographic variables (age, marital status, employment status, education, household income, and gender), questions about self-rated health, self-efficacy for PA and intention to be physically active, perceived health benefits of PA and barriers to PA (individual-level variables), social support for PA, and availability of facilities for PA were also included according to a social-ecological model. All the questions were rated in a Likert scale as described below.

2.6. *Outcome variable*

To obtain a dichotomous outcome variable, we calculated metabolic equivalent (MET)-hours according to the IPAQ scoring guidelines (*http://www.ipaq.ki.se/scoring.pdf*) (*16*). Outcome variable MET-hours were derived by multiplying total hours of vigorous PA, moderate PA, and walking with their respective MET. The values are as follows: vigorous PA (MET = 8.0), moderate PA (MET = 4.0), and walking (MET = 3.3). Thus MET-hours were calculated by the following method: MET-hours = (weekly total hours of vigorous PA × 8.0) + (total hours of moderate PA × 4.0) + (weekly total hours of walking × 3.3). As an index for PA, sufficient PA was defined as achieving a minimum of 840 MET minutes/week from any combination of walking, moderate-intensity or vigorous intensity activities. We used this definition to compare with a similar survey done in Malaysia (*8*).

2.7. *Description of explanatory variables*

2.7.1. *Individual level variables*

Intention to be physically active was assessed as extent to which the participants intended to be physically active during the next six months. Participants were asked to rate their intention on a seven point Likert scale where '1' was no intention at all and '7' was

fully intended to be physically active. Similarly, self-efficacy was assessed by asking how confident they felt about doing 30 minutes of moderate PA (3-4 days in a week) and 60 minutes of light PA each day. They were asked to rate this on a seven point Likert scale where '1' was not at all confident and '7' was very confident. Perceived health benefits were assessed by six items (*e.g.* improved physical attractiveness, prevention of heart disease, obesity, cancer, stress, diabetes, *etc.*). The participants were asked to rate their agreement with the statements on a seven point Likert scale where '1' was I do not agree and '7' was I agree very strongly. Barriers to PA were assessed by providing a list of eight items in which participants were asked "What prevents you from participating in regular PA?" (*e.g.* lack of time, energy, skills, motivation, *etc.*) The participants were asked to rate from '1' not at all important to '7' very important on a seven point Likert scale.

2.7.2. *Social and physical environment variables*

Participants were asked to rate a list of six factors that influence people to engage in PA (example: information about health and well being, planning daily schedule, professional advice and coaching, *etc.*). The rating was done on a seven point Likert scale from '1' as not important at all to '7' as very important. Availability of physical structures and types of infrastructure (*i.e.*, facilities for PA) surrounding their neighborhood was assessed by classifying them into four groups (*e.g.* jogging tracks, bicycle lanes, swimming pools, gymnasium, *etc.*). The participants were asked to report about availability of facilities for PA in their local communities as 'none at all', 'some', and 'many' which were scored as '1', '2', and '3', respectively.

2.8. *Data collection*

During the month of October 2011, e-mail invitations to participate were sent out to all the registered participants for the monthly UTAR opinion poll survey. E-mail provided a link to the survey with all the instructions included. The survey also provided an option for participants to invite their friends to participate in the survey. Consent was taken from the participants of the survey at the time of registration. Confidentiality and anonymity about the survey responses was assured for all the participants. All surveys were approved by the Research Ethics Committee of UTAR.

2.9. *Data analysis*

The data was extracted from the UTAR opinion poll survey web pages into Microsoft excel. The data set was converted into SPSS for labeling variables, recoding, and analysis. MET-hours were computed as explained above. MET-hours were re-coded to create sufficient PA and explanatory variables were also re-coded to combine some categorical variables. For individual, physical and social environmental factors (except self-rated health), we developed a total score by summing up individual responses given by each respondent on Likert scales explained above. Descriptive statistics were calculated for scores generated for individual, physical and social environmental factors and other continuous variables according to socio-demographic variables. Statistical tests of significance namely ANOVA, and independent samples *t*-test were used for continuous variables and chi square tests for categorical variables. By univariate analysis, unadjusted OR and their 95% CIs were calculated. To identify the factors associated with sufficient PA, explanatory variables with p-value < 0.05 were included in multivariate analysis to calculate adjusted OR and their 95% CIs. For all statistical tests a p-value of < 0.05 was considered as significant.

3. Results and Discussion

A total of 475 participants completed the survey. One participant's responses were incomplete and were excluded from analysis. Of these 253 (53.4%) were females while 221 (46.6%) were males. The mean age was 22.4 years (S.D. = 4.7) and median age was 21 years (inter-quartile range, 20-23). Most of the participants were studying full time (341, 72.4%), single (360, 75.9%) and had studied up to a bachelors degree (306, 64.6%) (Table 1).

Mean MET-hours was 31.36 (S.D., 52.19) and mean MET-minutes was 1881.91 (S.D., 3,131.96). The data was highly skewed (skewness, 3.8). Median MET-hours was 14.7 (IQR, 5.77-32.07) and median MET-minutes was 883.0 (IQR, 346.50-1924.25). Based on our operational definition, 242 (51.1%) of participants had done a sufficient amount of PA during the previous seven days. Mean MET-hours for those who had carried out sufficient and insufficient PA were 55.83 (S.D., 64.05) and 5.84 (S.D., 4.22), respectively, while median MET-hours were 31.42 (IQR, 21.83-60.70) and 5.65 (IQR, 2.2-9.48), respectively.

Men and participants who reported to have good/very good health had done more PA when measured in terms of MET-hours and as a proportion of sufficient PA both of which was statistically significant (Table 1). On univariate analysis, being male, self-perception of health as very good or good, positive intention, self-efficacy, perceived benefits of PA, social support, and availability of facilities for PA were associated with sufficient PA (Table 2). However, on multivariate analysis after adjustment for possible interactions between explanatory variables, participants who intended to be physically active (OR 0.75, 95% CIs 0.64, 0.88), and having higher confidence of doing PA (OR 0.91, 95% CIs 0.85, 0.97) were likely to have done sufficient PA during the last seven days. Participants

Table 1. Physical activity (MET-hours) by demographic variables of the study population

Variable	Mean (S.D.)	Number of participants	Mean level of PA (MET-hours)[†]	Sufficient PA (*n* (%)[€])
Age (years)	22.35 (4.7)			
≤ 20 years		181	31.6 (52.3)	89 (49.2)
21-24 years		203	33.8 (58.3)	109 (53.7)
≥ 25 years		46	25.4 (34.7)	44 (95.7)
Sex*				
Male		221	39.9 (63.3)	125 (56.6)
Female		253	23.9 (38.8)	96 (37.9)
Monthly household income				
< 2,001		324	31.1 (52.9)	160 (49.4)
2,001-4,000		108	34.8 (54.6)	61 (56.5)
> 4,000		42	25.8 (38.8)	21 (50.0)
Educational attainment				
Up to secondary		150	31.5 (46.6)	75 (50.0)
Bachelors		306	31.9 (56.0)	157 (51.3)
Masters and above		18	20.8 (19.3)	10 (55.6)
Occupation				
Studying full time		341	32.9 (54.5)	178 (52.2)
Working full time		113	28.9 (47.8)	55 (48.7)
Unemployed/part time work		20	22.9 (31.4)	9 (45.0)
Marital status				
Single		360	32.3 (53.5)	188 (52.2)
Into a relationship/married		114	28.5 (47.8)	54 (47.4)
Self-rated health				
Good/very good		168	38.6 (56.8)*	101 (60.1)*
Fair		241	28.9 (52.1)	117 (48.5)
Poor/very poor		65	21.6 (35.5)	24 (36.9)
Individual, Physical and social environmental factors				
Intention to do PA	4.43 (1.5)			
Self-efficacy for PA	8.42 (3.6)			
Perceived health benefits of PA	30.6 (8.9)			
Perceived barriers to PA	31.2 (9.2)			
Social support for PA	28.1 (8.3)			
Availability of facilities for PA	6.86 (1.8)			

* $p < 0.05$; † Non-parametric tests for comparison of means was used; € Chi square test was used.

who reported that many facilities were available for PA (OR 0.81, 95% CIs 0.73, 0.91) were more likely to have done sufficient PA (Table 3).

This pilot survey among young Malaysian adults showed that nearly half of them had done sufficient PA and nearly a quarter of them had done very minimal PA. Our results show that socio-demographic variables were not associated with PA, though being male, self-rated health as good, perceived benefits of PA, and social support for PA were associated with sufficient PA on univariate analysis but not associated with PA on multivariate analyses. On multivariate analysis, intention, self-efficacy, and availability of facilities were the strongest correlates of PA.

Results of our survey should be interpreted with caution against the limitations we had in our survey design and sample we surveyed. A purposive, non-representative sample used for the survey limits external validity of our results. Participant recruitment by online survey may have lead to selection bias. The correlates of PA from our cross-sectional survey lack temporal sequence thus limiting causal inference. Self-reported PA measures may have been over-reported about time spent doing PA. Moreover, we did not assess total PA without including time spent in separate domains like exercise or sport, recreational, occupational and transportation activities. Despite these limitations our survey may serve as a benchmark for further studies to assess interactions among individual, social and physical environmental factors.

The PA level reported in our survey is much lower than those reported from high income nations like Canada, USA, Belgium, and Sweden (*10-13,17*). However, such comparisons are limited because all these studies used varied instruments, and surveys were done among different age groups (*10-13,17*). There is a relative lack of literature about PA from the southeast Asia region except a few from Thailand and a report from five Asian countries (India, Bangladesh, Vietnam, Indonesia, and Thailand) which assessed PA using the Global Physical Activity Questionnaire version 2 (GPAQ-2) and another survey from six Asia-Pacific countries (*18-20*). A national survey on metabolic syndrome collected data about PA but not using IPAQ (*7*). One survey among school children in Selangor state, Malaysia used a short version of IPAQ to assess PA and reported that 20.8% had < 600 MET-minutes/week (*8*). Thus level of PA measured was

Table 2. Univariate analysis of demographic, individual, social and physical environmental factors with sufficient PA

Variable	Unadjusted odds ratio	95% Confidence intervals (CIs)	*p*-value
Age (years)			
≤ 20 years	1		
21-24 years	0.83	0.56, 1.25	0.376
≥ 25 years	1.01	0.61, 1.68	0.965
Sex			
Male	1		
Female	1.51	1.05, 2.18	0.025
Monthly household income			
< 2,001	1		
2,001-4,000	0.75	0.48, 1.17	0.20
> 4,000	0.98	0.51, 1.86	0.94
Educational attainment			
Up to secondary	1		
Bachelors	0.95	0.64, 1.40	0.79
Masters and above	0.80	0.29, 2.14	0.66
Occupation			
Studying full time	1		
Working full time	1.15	0.75, 1.76	0.52
Unemployed/part time work	1.34	0.54, 3.30	0.53
Marital status			
Single	1		0.37
Into a relationship/married	0.82	0.54, 1.26	0.57
Self-rated health			
Good/very good	1		
Fair	1.61	1.07, 2.38	0.021
Poor/very poor	2.56	1.43, 4.65	0.002
Individual, Physical and social environmental factors			
Intention to do PA	0.64	0.56, 0.74	< 0.001
Self-efficacy for PA	0.82	0.78, 0.87	< 0.001
Perceived health benefits of PA	0.95	0.93, 0.97	0.000
Perceived barriers to PA	1.19	0.94, 1.52	0.16
Social support for PA	0.96	0.94, 0.98	0.001
Availability of facilities for PA	0.79	0.71, 0.88	< 0.001

Table 3. Multivariate analysis of demographics, individual, social and physical environmental factors for sufficient PA

Variable	Unadjusted odds ratio	95% Confidence intervals (CIs)	*p*-value
Sex			
Male	1		
Female	1.330	0.89, 1.99	0.17
Self-rated health			
Good/very good	1		
Fair	1.139	0.73, 1.77	0.565
Poor/very poor	1.600	0.82, 3.12	0.167
Individual, Physical and social environmental factors			
Intention to do PA	0.75	0.64, 0.88	< 0.001
Self-efficacy for PA	0.91	0.85, 0.97	0.007
Perceived health benefits of PA	0.98	0.96, 1.01	0.204
Social support for PA	0.98	0.96, 1.02	0.330
Availability of facilities for PA	0.81	0.73, 0.91	0.001

not comparable across various studies. Thus there is a need for use of a standardized instrument and uniform criteria to measure PA (*21*).

Correlates of PA we identified according to the social-ecological model assume importance to design interventions to improve PA among young adults. From our study males were physically more active than females; which is similar to previous studies (*19,20*). Perceived poor health has been associated with lower PA in some studies (*22-24*). However, our study and a study from 15 member states of the European Union have reported that better self-rated health was associated with a higher level of PA (*13,24,25*). Self-efficacy, intention, and facilities for PA were strongly associated with higher PA in our study. These factors were consistently associated with higher PA in many studies done in different populations (*4,11,12,17,26-33*). Though countries and socio-ecological milieu where the participants reside may vary widely, we infer from our results that these three factors are very important to

carry out PA. For instance, intention to do PA may have a proximal goal which would provide self incentives and act as a guide to healthy habits (*34*). Perceived self-efficacy is another vital influencing factor on PA as it measures confidence level in one's own ability which is necessary for persistence of healthy behavior (*34*). Health promotion interventions aimed at increasing PA level should target self-efficiency and intention for PA. These alone are not sufficient without the availability of physical structures for PA. Our study provides evidence for this as people who perceived that availability of physical facilities for PA as 'some' or 'many' were likely to have done sufficient PA. A meta-analysis has also reported that a perceived physical environment was associated with PA (*28*). However, availability alone cannot predict indulgence in PA but general condition of the neighborhood is also important for a decision to indulge in PA (*26*). Factors such as traffic conditions, pavements for pedestrians, street lights, weather conditions (temperature, rain), street dogs, and street crime also determine suitability of the neighborhood for indulgence in PA (*4,25,35*). Social support, perceived health benefits of PA and perceived barriers to PA were not significant in our survey on a small sample due to lack of power to detect these differences or non-representativeness of the sample we surveyed. However, these were shown to be important factors determining PA and varied across socio-demographic variables according to a study from Canada and Malaysia (*8,9,12*).

In conclusion, results of our exploratory survey suggest that level of PA may not be sufficient among young adults. Factors such as intention, self-efficacy, and facilities for PA which were significant may be considered while planning health promotional strategies. Larger surveys at the national level using standardized instruments and inclusion of factors according to the socio-ecological model would provide better understanding about correlates of PA.

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Brief Report

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Drug susceptibility pattern of *Mycobacterium tuberculosis* isolates from patients of Category-II failure of pulmonary tuberculosis under directly observed treatment short-course from north India

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Summary **The major contributing factors for the causation of treatment failure in cases of pulmonary tuberculosis under Category-II directly observed treatment short-course treatment (DOTS) are treatment after default, poor treatment compliance, and development of multi-drug resistant (MDR) tuberculosis. The objective of the present study is to find out the demographic profile and drug susceptibility pattern in Category-II failure patients of pulmonary tuberculosis under Revised National Tuberculosis Control Programme (RNTCP) of India. Two hundreds and twenty four patients with Category-II treatment failure of pulmonary tuberculosis were enrolled from Department of Pulmonary Medicine, at Chatrapati Sahuji Maharaj Medical University, UP, Lucknow, India, from August 2003 to July 2008. Their complete bacteriological assessment in terms of sputum smear for acid-fast bacilli, culture for *Mycobacterium tuberculosis* and drug sensitivity pattern were done in the Department of Microbiology. Among 224 patients, 16 (7.1%) patients were lost to follow-up and the final analysis was done among 208 (92.8%) cases. The reasons for inclusion of these 224 cases in the Category II regimen were treatment failure in the previous regimen (*n* = 75, 33%), default in 57% (*n* = 129 cases), and relapse in 8.9% (*n* = 20 cases). Among 208 patients, culture was positive in 170 (81.7%) cases, negative in 17 (8.1%) cases and contaminated in 21 (10%) cases. The drug sensitivity pattern of culture positive cases of Category-II failure patients revealed that, 58.2% (*n* = 99) had MDR tuberculosis and 40.5% (*n* = 69) were resistant but were non-MDR tuberculosis and 1.1 % (*n* = 2) cases were sensitive to all first line antituberculosis drugs.**

Keywords: Category-II, failure, MDR, tuberculosis, RNTCP

1. Introduction

India accounts for nearly one-third of the global burden of tuberculosis and two-thirds of the total cases in South-East Asia. Nearly 40 percent of the Indian population is infected with the tuberculosis bacillus. Increasing awareness of the rising global rates of multi-drug resistant (MDR) tuberculosis has led to a concerted international effort to confront this disease, particularly in India with the high incidence of tuberculosis. As per the latest estimates from the State representative drug resistance surveillance (DRS) survey of India in Gujarat states and various district level DRS studies, the prevalence of MDR tuberculosis in new smear positive

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pulmonary tuberculosis cases is < 3% and 12% to 17% among smear positive previously treated pulmonary tuberculosis cases (*1*).

In India, the Revised National Tuberculosis Control Programme (RNTCP), adopting the directly observed treatment short-course (DOTS) strategy advocated by the World Health Organization (WHO), was implemented in 1993 in the country and has been scaled up rapidly since mid 1998. In India, all RNTCP treatment regimens are given three times weekly on alternate days. Based on a stringent diagnostic algorithm and history of previous tuberculosis treatment, the diagnosed cases under RNTCP are classified as 'New' and are put on the Category-I regimen. Re-treatment cases *i.e.*, those who give a history of previous tuberculosis treatment of more than one month, are put on the Category-II regimen. This category comprises smear positive 'Failures', 'Relapses', 'Treatment after default' and 'Others'.

Under RNTCP, treatment failure is defined as, a patient who, while on treatment, remained or became again smear positive five months or later after commencing treatment. Category-II treatment failure patients, where chances of MDR tuberculosis are very high, remain the most difficult problem in the management of tuberculosis (*2,3*). In various studies, among Category-II failure patients, the prevalence of MDR tuberculosis varied from 4% to 80% (*4-8*).

In the present study we have attempted to determine the demographic profile and drug susceptibility pattern in Category-II patients of pulmonary tuberculosis, under RNTCP so that we can identify MDR tuberculosis cases early and reduce their transmission by starting second line treatment for MDR tuberculosis.

2. Materials and Methods

2.1. *Study design*

Prospective, cohort study of Category-II failure patients of pulmonary tuberculosis under RNTCP, between August 2003 and July 2008, referred from various districts of Uttar Pradesh (India) were studied.

2.2. *Setting*

The current study was set in Department of Pulmonary Medicine & Department of Microbiology, CSM Medical University, Lucknow, India (WHO recommended Intermediate Reference laboratory).

2.3. *Criteria for selection of patients*

Inclusion criteria: (*i*) All tuberculosis patients, who failed on Category-II DOTS treatment, (*ii*) Age 15-70 years. Exclusion criteria: Patients who already had taken the Category-IV regimen for treatment.

2.4. *Patients clinical evaluation*

A detailed history of previous treatment was taken and a thorough clinical examination was done with a structured questionnaire. Chest radiograph, liver and renal function tests, and blood sugar estimation were also done. Screening for human immunodeficiency virus (HIV) infection was done in all the patients after consent. The diagnosis of smear positive pulmonary tuberculosis was done as per the RNTCP diagnostic algorithm.

2.5. *Sample collection*

All patients were directed to collect the early morning sputum specimen in a sterilized wide-mouthed bottle with a tightly fitting cork stopper. Sputum was sent for smear for acid-fast bacilli (AFB) and culture/sensitivity, on three consecutive days. All sputum samples were transported to the Department of Microbiology (WHO recommended IRL laboratory), as soon as possible after collection. The Department of Microbiology has been identified as an Intermediate Reference laboratory for quality control in RNTCP under the government of India/WHO, World Bank.

2.6. *Laboratory method*

Homogenization and concentration of specimen was done by modified petroff's method and staining for acid fast bacilli was done by the Zeihl Neelsen Method as described by Cruickshank (1965) (*9*).

2.7. *Culture examination*

Conventional solid egg-based Lowenstein-Jensen (LJ) media was used for primary culture. All cultures were incubated at 35-37°C until growth was observed or discarded as negative after eight weeks. Slopes that were grossly contaminated also were discarded.

All cultures were examined 48-72 h after inoculation to detect gross contaminants and thereafter cultures were examined weekly, up to 8 weeks. The colonies of *Mycobacterium tuberculosis* were defined as rough, crumbly, waxy, non-pigmented (buff colored). Negative cultures were defined as no growth after 8 weeks. The positive cultures showing AFB were identified as *M. tuberculosis* based on the results of growth on LJ medium containing *p*-nitrobenzoic acid and niacin tests (*10,11*). The presence of AFB in primary cultures was confirmed by Zeihl Neelsen staining.

2.8. *Drug susceptibility tests*

All *M. tuberculosis* cultures were subjected to drug susceptibility tests for isoniazid (H), rifampicin (R), ethambutol (E), streptomycin (S), and pyrazinamide (Z)

by the economic version of proportion method as per the International Union against Tuberculosis and Lung Disease (IUATLD), Manual for the National Laboratory Network (*4*). The critical proportion for declaring a strain as resistant to the drugs was 1%.

2.9. *Ethical clearance*

The Ethical committee of our university approved the present study.

2.10. *Definition used in present study*

(*i*) Category-II failure: If the patient has completed more than 5 months of Category-II treatment and remained sputum positive.

(*ii*) MDR tuberculosis case: Those that have a sputum positive culture and whose tuberculosis is due to *M. tuberculosis* that are resistant *in vitro* to at least isoniazid and rifampicin (the culture and drug susceptibility test's result being from an RNTCP accredited laboratory).

(*iii*) Drug sensitive: Absence of resistance to any of the first line antituberculosis drugs, *e.g.* streptomycin, rifampicin, isoniazid, ethambutol, and pyrazinamide.

(*iv*) Monoresistance: Resistance to only 1 drug from the first line antituberculosis drugs. Polyresistance: Resistance to at least two or more drugs excluding the R and H combination.

3. Results and Discussion

A total of 224 patients, which failed on Category-II DOTS treatment, were included in the present analysis even though it was planned to collect a sputum specimen from all patients, unfortunately 16 (7.1%) patients were lost to follow-up. So the final analysis was done among, 208 (92.8%) cases. The reasons for inclusion of these 224 cases in the Category-II regimen failure cases were treatment failure in 75 cases (33%), treatment after default of previous treatment in 129 cases (57%), and relapse in 20 cases (8.9%). Among 208 patients, cultures were positive in 170 (81.7%) cases, negative in 17 (8.1%) cases, and contaminated in 21 (10%) cases. Among 170 patients, who produced positive cultures, 2 (1.1%) harbored sensitive bacilli, 99 (58.2%) had an MDR tuberculosis pattern, and 69 (40.5%) cases were resistant but a non-MDR tuberculosis pattern.

There was a preponderance of males, (n = 141, 68.2%) as compared to females (n = 67, 32.3%). Of all, 96 (46%) cases were from rural areas and 112 (54%) from urban areas. 79.4% of patients were from age group 15-35 years and from lower socioeconomic status. All patients tested were sputum smear positive for AFB and percentage grading as +3, +2, +1, and scanty were 41.7%, 27%, 23%, and 8.2%, respectively. Of all, 43 cases were current smokers and 11 of them had MDR tuberculosis and 79 cases were ex-smokers, and 38 of them had MDR tuberculosis. Fifty-seven (27.4%) cases had a history of alcohol intake and 11 of them had MDR tuberculosis. Diabetes mellitus was associated with 18 (8.6%) cases of Category-II failure and 4 of them had MDR tuberculosis. Twenty-three Category-II failure patients were infected with HIV and five of them had MDR tuberculosis.

Before Category-II treatment, 76 cases (36.5%) had taken DOTS treatment and 30 of them had MDR tuberculosis, while 132 (63.5%) cases had taken treatment from private practitioners and 69 of them had MDR tuberculosis. The detailed analysis is given in Tables 1 and 2.

One of the most important landmarks in the history of tuberculosis control is the introduction and implementation of the RNTCP based on the globally recommended DOTS strategy that was implemented in India in a phased manner since 1993. W.H.O. recommends retreatment with the Category-II Anti Tuberculosis Treatment regimen (2H3R3Z3E3S3 + 1H3R3Z3E3 + 5H3R3E3) for patients with relapse, treatment failure or treatment after interruption (*5*). The treatment success rate for previously treated patients with the re-treatment regimen, *i.e.,* Category-II was low 71%. The probable reasons for Category-II treatment failure are treatment after default, poor treatment compliance and development of MDR tuberculosis.

The present study revealed that 58.2% patients of Category-II failure had MDR tuberculosis and 40.5% cases had non-MDR tuberculosis.

Table 1. Demographic profile of MDR tuberculosis cases (n = 99/170*)

Item	Number	Percentage
Sex		
Male	65	38.2%
Female	34	20.0%
Age wise distribution		
< 15-24 years	33	19.4%
25-34 years	45	26.4%
35-45 years	20	11.7%
> 45 years	1	0.5%
Residence		
Rural	41	24.1%
Urban	58	34.1%
H/O Smoking		
Current smokers	11	6.4%
Ex-smokers	38	22.3%
Non-smoker	50	29.4%
H/O alcohol intake		
Present	11	6.4%
Absent	88	51.7%
H/O diabetes mellitus		
Present	4	2.3%
Absent	95	55.8%
H/O HIV		
Present	5	2.9%
Absent	94	55.9%
H/O prior treatment		
By private practitioner	69	40.5%
By the Government (DOTS)	30	17.6%

* Total numbers of culture positive patients, *i.e.,* 170.

Table 2. Drug susceptibility pattern of drug resistant patients (*n* = 170)

Item	Number of cases	
	Number	Percentage
Any resistance		
Isoniazid (H)	131	77.9
Rifampicin (R)	116	69
Ethambutol (E)	86	51.1
Streptomycin (S)	93	55.3
Pyrazinamide (Z)	46	27.3
Monoresistance		
Isoniazid (H)	5	2.9
Rifampicin (R)	2	1.1
Ethambutol (E)	3	1.7
Streptomycin (S)	4	2.3
Pyrazinamide (Z)	1	0.5
Multi-drug resistance		
H + R	9	4.7
H + R+Z	2	0.5
H + R + E	14	8.3
H + R + S	26	15.4
H + R + E + S	19	11.3
H + R + E + Z	2	1.1
H + R + S + Z	1	0.5
H + R + E + S + Z	16	9.5
Other patterns		
H + E	2	1.1
H + S	6	3.5
R + Z	6	3.5
R + S	4	2.3
S + Z	3	1.7
E + S	1	0.5
H + E + S	14	8.3
R + E + S	2	1.1
S + E + Z	7	4.1
S + R + Z	4	2.3
S + H + Z	5	2.9
S + R + E +Z	1	0.5

A study from Malawi analyzed data of 748 patients, who had taken the retreatment regimen and of which only 307 (41%) patients had sputum sent for culture and drug sensitivity tests. Fifty-three percent specimens grew organisms resembling *M. tuberculosis*. Of the positive cultures, 81% showed full sensitivity to all drugs tested, 15% showed non-MDR tuberculosis, and 4% showed a MDR tuberculosis pattern (*6*).

Another study from India reported the drug susceptibility profile of 'failures' from the Category-II regimen in pulmonary tuberculosis. Among 431 patients who produced positive cultures, 59% harbored sensitive bacilli, 30% were resistant but non-MDR bacilli, and 11% had MDR tuberculosis (*7*).

Another study from the Netherlands was done to determine acquired drug resistance among failure and relapse cases, in 2,901 patients of smear-positive tuberculosis, who had taken the 2SHRZ/6HE treatment regimen. They concluded that of the failure cases to Category-I regimen (2SHRZ/6EH), 80% had MDR-tuberculosis and among relapse cases, 8% had MDR-tuberculosis (*8*).

Another study, which included data from six countries or areas (Dominican Republic, Hong Kong, Italy, Ivanovo, the Republic of Korea, and Peru), analyzed 6,402 culture positive tuberculosis cases of which, 86% were new cases and 14% were retreatment cases. Among retreatment cases 44.5% were drug resistant and 43.6% had an MDR pattern, while a non-MDR pattern was observed in 56.6% (*12*).

Another study from Morocco was done to evaluate the prevalence and patterns of drug resistance of *M. tuberculosis* isolates from patients with chronic tuberculosis. They showed that 89.3% of the strains were resistant to at least one anti-tuberculosis drug, 5.7% were resistant to only one drug and 83.5% to two or more drugs, with 76.2% of isolates resistant to isoniazid and rifampicin (*13*).

Another study was done among Category-I and retreatment failure patients of pulmonary tuberculosis and they observed MDR tuberculosis, in 1% and 39.7% of cases, respectively (*14*).

Another study from India among 271 new smear and culture positive patients, who had taken Category-I, it was observed that initial drug resistance to any drug was 27% with the MDR pattern being 2.2%. Few studies have reported very high rates of MDR tuberculosis in patients who fail the Category-I regimen. A case-control study from Peru reported that among failures to the Category-I (2EHRZ/4R2H2) treatment, 75% had MDR tuberculosis. Treatment failure in urban Lima has been identified as a strong predictor of MDR tuberculosis (*15*). Another study from India reported a high proportion of MDR tuberculosis strains in both previously untreated (24%) and treatment-failure cases (41%). They also concluded that among new cases, resistance to 3 or 4 drug combinations (amplified drug resistance) including isoniazid (H) and rifampicin (R), was greater (20%) than resistance to H and R alone (4%) at any point in time (*16*).

Another study from India was done in two districts to measure the levels and pattern of resistance to anti-tuberculosis drugs among "newly diagnosed" sputum smear positive pulmonary tuberculosis cases. They further stated that MDR was 0.7% and 3.0%, respectively, in the Mayurbhanj and Hoogli districts (*17*). Another study from India reported a drug susceptibility profile of Category-I failure cases and found that 17% of cases had an MDR pattern, while 30% of cases had a non-MDR pattern (*18*).

The percentage of MDR tuberculosis among supervised Category-II treatment and un-supervised Category-II treatment were 33.4% and 72.4%, respectively.

The present study also highlighted that 63.5% of cases of Category-II failure had not taken DOTS treatment earlier and were treated by private practitioners. In addition to the above, the present study also highlighted the percentage of MDR tuberculosis among DOTS Category-II and non-DOTS Category-II patients were 38.3% and 61.6%, respectively. A study from India also reported that 32% of cases of Category-II failure were from the private sector (*19*).

There is concern regarding the effectiveness of the Category-II regimen for re-treatment cases especially for failures. A study from Siberia showed that only 46% of the patients could be declared cured on the basis of sputum smear microscopy at the end of the therapy (*20*). They also reported that this five drug regimen of Category-II of WHO is inadequate for a re-treatment regimen. The author himself reported a clinical profile of 3 Category-II failure cases earlier and found that 2 of them had an MDR pattern and strongly advocated that Category-II appears good for relapse and treatment for default types of patients. But for the treatment failure group, is this Category adequate (*21*) ? To answer this question a controlled clinical trial is required on a large number of such types of patients.

A major limitation of our study is that sampling is not representative of the general pool of Category-II failure patients in the community. It is a reflection of the drug sensitivity pattern in patients being referred to a tertiary care center; nevertheless we took consecutive samplings over the course of the study.

In conclusion, the present study highlights that more than half of Category-II failure patients have MDR tuberculosis and the RNTCP policy in India of treating all re-treatment cases with the WHO recommended re-treatment regimen (*i.e.,* Category-II) may be adequate except for the MDR tuberculosis patients. Drug susceptibility tests should be done for patients who remain sputum smear positive during the retreatment period and appropriate regimens should be started as early as possible for a better treatment outcome and to reduce transmission of drug resistant tuberculosis.

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Original Article

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A lifetime experience of violence and adverse reproductive outcomes: Findings from population surveys in India

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Summary **Intimate partner violence (IPV) is a global public health issue that threatens the reproductive health of women. Despite a growing demand for research on the potential threat of IPV in relation to adverse reproductive outcomes, there have been no population-based studies of India. The current study analyzed the National Family Health Survey 3, which contained detailed information on types of violence in relation to the single question of pregnancy outcomes. The dataset was used to assess the association between a lifetime experience of IPV and terminated pregnancies among married Indian women. Multiple logistic regression analysis was then used to assess the association between these variables, controlling for socio-demographic characteristics. Results showed that 39.6% of Indian women have experienced violence by their husbands, while 18.3% of women have terminated a pregnancy during their lifetimes. The odds ratio of a terminated pregnancy among women who had experienced any type of partner violence was 1.62 (95% CI (confidence interval) = 1.51-1.73). All combinations of violence except a combination of emotional and sexual violence were associated with an increased risk of a terminated pregnancy. These results suggest that prevention of IPV would reduce the high incidence of terminated pregnancies, thus improving maternal health in India.**

Keywords: Intimate partner violence, terminated pregnancy, India, national sample

1. Introduction

Over the past decades, intimate partner violence (IPV) has been globally recognized as a serious public health issue that threatens the health and rights of women. Its prevalence has been shown to range from 15% to 71% worldwide (*1*). IPV usually refers to "any behavior within an intimate relationship that causes physical, psychological, or sexual harm to those in the relationship" (*2*). It often occurs among people of lower economic status, lower educational attainment, and those who are young (*2-4*). Health issues for women in relation to IPV include injuries, chronic pain syndrome, and substance abuse, depression, and suicide attempts (*2,5*). Increasing evidence points to an association between IPV and several reproductive health outcomes: low-birth-weight (*6*), sexually transmitted infections such as HIV/AIDS (*2,7*), less frequent contraceptive use (*8*), and adverse pregnancy outcomes (*9-13*).

Recent evidence has suggested the importance of IPV prevention to reduce the incidence of terminated pregnancies (*11-13*). A terminated pregnancy was deemed to be any pregnancy that was miscarried (spontaneous abortion), aborted, or ended in a stillbirth. Although there is the possibility that termination of a pregnancy may influence the way a husband treats his wife, the existence of violence underlying termination of a pregnancy is an important issue that should be investigated and acknowledged in societal and clinical settings.

However, evidence supporting an association between IPV and adverse pregnancy outcomes has been limited by the scope of the studies being either hospital- or community-based in India. India is estimated to have 39 stillbirths per 1,000 births and 6 million induced abortions annually (*14-16*). Despite government

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efforts to permit legal abortion in a broad range of circumstances, illegal abortions carried out by non-qualified health providers and those based on the sex of the infant account for 90% of abortions and are believed to be responsible for 12-20% of all maternal deaths in India (*14-16*). Therefore, causes and risk factors must soon be identified so that policymakers can improve maternal morbidity and mortality in India.

The present study aimed to determine the association between IPV and pregnancy termination using a national sample of Indian women from the National Family Health Survey (NFHS). This dataset was used to investigate the relationship between women's reports of a lifetime experience of physical, sexual, and emotional IPV and having terminated a pregnancy among a national sample of married Indian women.

2. Materials and Methods

2.1. *Data*

Data for the present study were from NFHS-3. The survey, conducted between November 2005 and August 2006, included a domestic violence module that provided detailed information on violence. The survey featured a nationwide sample designed to provide indicators on fertility, family welfare, and health at national and state levels and was conducted by the Ministry of Health and Family Welfare, Government of India in collaboration with the International Institute for Population Sciences (IIPS) and Macro International, Maryland, USA. The NFHS covered all of the states of India using a multi-stage sampling method. Further details on the sampling design and selection method are described elsewhere (*17,18*).

2.2. *Participants*

In NFHS-3, the questionnaire consisted of two parts: a women's questionnaire that investigated women's socio-demographic status, maternal health, contraceptive use, and other aspects and a domestic violence module that asked about a lifetime experience of violence. The women's questionnaire was given to women aged 15-49. As a safety precaution, however, only one woman per household was asked to complete the domestic violence module. Additionally, the questionnaire was not administered in cases where privacy could not be ensured. Of the 124,385 women responding to the women's questionnaire, 83,703 were selected to answer the domestic violence module. The module covered 67% of the entire NFHS-3 sample in the women's questionnaire.

Women were included in this study if they were: 1) de jure residents of India; 2) currently married; 3) had data available on both IPV and pregnancy termination. Ultimately, this study had 63,473 participants.

2.3. *Measurements*

The questionnaires for each state were prepared in the principal language of the state and English. In NFHS-3, a question asked whether respondents had ever attempted to terminate a pregnancy resulting in a non-live birth. Types of non-live births were not distinguished. Experience of domestic violence was approached comprehensively using the Domestic Violence Module. This module adopted the shortened and modified version of the Conflict Tactics Scale (*19*). Three types of IPV were measured: physical, sexual, and emotional. Physical and sexual violence were measured using a set of questions and categorized as follows: 1) slapping; 2) twisting of the arm or pulling of hair; 3) pushing, shaking, or throwing something at the respondent; 4) punching; 5) kicking, dragging, or beating; 6) choking or burning; 7) threatening with a weapon; 8) forcing the respondent to have sex; and 9) forcing the respondent to perform degrading sexual acts. Women responding "yes" to any type from 1) to 7) were considered to have experienced physical violence and those who answered "yes" to 8) to 9) were considered to have experienced sexual violence. Emotional violence was assessed by whether a husband had ever subjected the respondent to: 1) humiliation; 2) threats to harm the respondent or someone close to her; and 3) insults. Respondents answering "yes" to any of these were regarded as having been emotionally abused. The scale of internal consistency in this study was measured by Cronbach's alpha and was 0.82.

To identify the impact of types of violence on pregnancy termination, violence was assessed through a variety of categories other than that of "any experience of violence," including: 1) only physical violence; 2) only sexual violence; 3) only emotional violence, 4) a combination of physical and sexual violence; 5) a combination of physical and emotional violence; 6) a combination of sexual and emotional violence; and 7) all types of violence. The categories were mutually exclusive.

In addition to a history of violence and terminated pregnancies, socio-demographic characteristics were selected based on previous studies to examine any association with IPV and terminated pregnancies. These characteristics included age (classified as 15-24, 25-34, and 35-49), the respondent and partner's education (none, primary, secondary, and higher, with the additional classification of 'unknown' for partner's education), place of residence (urban or rural), religion (Hindu, Muslim, and other), standard of living index (classified based on household assets, according to three quintiles, as low, medium, and high based on household assets), type of caste or tribe (scheduled caste, scheduled tribe, other backward classes, and none) and parity (number of live-born children) (none, 1-2, 3-4, and ≥ 5).

2.4. *Statistical analysis*

To account for complexity of the survey design, all analysis was done using Stata ver.9 (StataCorp, College Station, Texas). In this analysis, sampling weight was considered for selection probability and non-response rates and for primary sampling units and strata. Since only one woman per household was selected for the domestic violence module, domestic violence weights were used instead of sampling weights.

In this study, the independent variable was having experienced any type of IPV while the dependent variable was any termination of a pregnancy. Socio-demographic characteristics and experiences with IPV were first cross-tabulated and then a chi-squared test was performed to estimate the simple association of these variables. The significance level was set at less than 0.05. Next, the association between IPV and a terminated pregnancy was assessed using the adjusted odds ratio (AOR) according to multiple logistic regression analysis with a 95% confidence interval (95% CI). All socio-demographic characteristics were considered to be confounding variables.

Since there were few missing data for each variable in relation to the total number of responses, these missing data were believed to have little influence on outcomes. Thus, a limited number of responses with missing data were excluded from the calculations. Responses missing data on the respondent's education (n = 4), partner's education (n = 110), religion (n = 82), caste or tribe (n = 2,321), and standard of living index (n = 1,430) were excluded from the total of 63,473. Due to overlap of missing data among the respondents, the ultimate sample size for the analysis was 59,674 women.

2.5. *Ethical considerations*

Survey procedures and protocols for NFHS-3 were reviewed and approved by the ORC Macro Institutional Review Board and the International Institute for Population Sciences. Interviewers obtained informed consent from each respondent before the interview. For the Domestic Violence Module, special training was provided to every interviewer to ensure the respondent's privacy and confidentiality.

This study is based on secondary data analysis. Permission for the use of data was obtained from ORC Macro, Inc. in July 2008.

3. Results

3.1. *Socio-demographic factors and IPV patterns in NFHS-3*

Of the total of 59,674 female respondents, most were aged 35-49 (38.8%), categorized as having a high standard of living (41.0%), and belonged to a backward class (40.7%) (Table 1). As regards experiences with IPV, 39.6% of respondents reported having experienced IPV. Increases in the respondent and partner's education and standard of living were associated with less IPV. Increased parity was associated with an increase in IPV. Furthermore, IPV was more prevalent among Muslim women than Hindu women.

3.2. *Likelihood of a terminated pregnancy and its association with IPV according to NFHS-3*

According to this study, 18.3% of Indian women had terminated a pregnancy during their lifetime (Table 2). Women who had been exposed to any form of IPV (22.2%) were more likely to have terminated a pregnancy. Multiple logistic regression analysis showed that having terminated a pregnancy remained significant among these abused women even after controlling for socio-demographic factors (AOR = 1.62, 95% CI 1.51-1.73) (Table 3). A similar result was also obtained when controlling for different forms of violence and socio-demographics. Exposure to all forms of violence, with the exception of a combination of sexual and emotional violence, increased the odds ratio of having terminated a pregnancy (only physical: AOR = 1.55, 95% CI 1.42-1.68; only sexual: AOR = 1.63, 95% CI 1.30-2.05; only emotional: AOR = 1.22, 95% CI 1.00-1.49; physical and sexual violence: AOR = 2.23, 95% CI 1.91-2.60; physical and emotional violence: AOR = 1.52, 95% CI 1.35-1.70; all types of violence: AOR = 2.07, 95% CI 1.78-2.42). The standard of living index was the only covariate not associated with a terminated pregnancy.

4. Discussion

The current results revealed a clear association between IPV and having terminated a pregnancy among the Indian population. Indian women who had experienced partner violence were more likely to have terminated a pregnancy. Although IPV was more prevalent among the most disadvantaged, the association between IPV and termination of a pregnancy was observed through the entire population, irrespective of standard of living.

The main characteristic of NFHS-3 is the association of each combination of violence with termination of a pregnancy. With the exception of the combination of sexual and emotional violence, there was a statistically significant association between all forms of partner violence and terminated pregnancies among Indian women. Plausible explanations of these findings are that women tend to lose autonomy over their sexual lives, regardless of their standard of living, so they experience more unwanted pregnancies (*11,20,21*) and lose reproductive control (*8,22,23*) in addition to the direct physical harm that they suffer in abusive relationships.

Table 1. Sociodemographic factors and intimate partner violence (IPV) patterns among Indian women according to NFHS-3

Sociodemographic characteristics	*n* (%)	No IPV (%)	Any IPV (%)	*p*-value
Total	59,674 (100.0)	38,943 (60.4)	20,731 (39.6)	
Age				
15-24	11,672 (23.0)	7,787 (63.6)	3,885 (36.4)	< 0.001
25-34	25,947 (38.2)	16,759 (59.3)	9,188 (40.7)	
35-49	22,055 (38.8)	14,397 (59.6)	7,658 (40.4)	
Residence				
Urban	26,461 (31.2)	18,402 (68.1)	8,059 (31.9)	< 0.001
Rural	33,213 (68.8)	20,541 (56.9)	12,672 (43.1)	
Maternal education				
No education	23,502 (48.2)	12,705 (50.7)	10,797 (49.3)	< 0.001
Primary	9,142 (15.1)	5,520 (58.2)	3,622 (41.8)	
Secondary	21,733 (30.9)	16,050 (71.6)	5,683 (28.4)	
Higher	5,297 (5.7)	4,668 (87.5)	629 (12.5)	
Partner's education				
No education	13,315 (26.7)	7,127 (49.9)	6,188 (50.1)	< 0.001
Primary	9,418 (16.5)	5,305 (52.8)	4,113 (47.2)	
Secondary	28,107 (44.9)	19,316 (64.9)	8,791 (35.1)	
Higher	8,509 (11.4)	7,033 (78.8)	1,476 (21.2)	
Unknown	325 (0.6)	162 (51.3)	163 (48.7)	
Religion				
Hindu	45,730 (83.1)	29,620 (60.5)	16,110 (39.5)	< 0.001
Muslim	6,370 (11.5)	3,784 (56.0)	2,586 (44.0)	
Other	7,574 (5.34)	5,539 (67.7)	2,035 (32.3)	
Standard of living index				
Low	12,679 (25.6)	6,497 (47.4)	6,182 (52.6)	< 0.001
Medium	19,236 (33.5)	11,503 (56.1)	7,733 (43.9)	
High	27,759 (41.0)	20,943 (72.0)	6,816 (28.0)	
Caste or tribe				
Scheduled caste	10,549 (19.2)	5,842 (51.5)	4,707 (48.5)	< 0.001
Scheduled tribe	8,036 (8.5)	5,243 (53.4)	2,793 (46.6)	
Other backward classes	19,777 (40.7)	12,393 (59.7)	7,384 (40.3)	
Other	21,312 (31.6)	15,465 (68.6)	5,847 (31.4)	
Parity				
0	4,894 (9.1)	3,740 (71.8)	1,154 (28.2)	< 0.001
1-2	26,196 (39.1)	18,695 (67.1)	7,501 (32.9)	
3-4	19,753 (33.1)	11,930 (56.9)	7,823 (43.1)	
≥ 5	8,831 (18.8)	4,578 (47.1)	4,253 (52.9)	

Table 2. Prevalence of pregnancy termination and its association with IPV by type according to NFHS-3

Type of violence	National (*n* = 59,674)
	Terminated pregnancy *n* (%)
Total	10,835 (18.3)
No violence (*Ref.*)	6,010 (15.8)
Any type of violence†	4,825 (22.2)***
Only physical†	2,302 (21.5)***
Only sexual†	185 (21.7)**
Only emotional†	298 (18.2)
Physical and sexual†	496 (27.8)***
Physical and emotional†	958 (20.9)***
Sexual and emotional†	38 (19.4)
All types†	548 (26.1)***

† Compared to no violence; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

The association was greater for a combination of physical and sexual violence and all types of violence than for physical or emotional violence alone or the combination of physical and emotional violence. This may be because physical and sexual violence are directly associated with physical injuries and/or unwanted pregnancy. However, the combination of sexual and emotional violence was the only type that was not associated with a terminated pregnancy. This result has to be considered carefully because very few respondents answered "yes" to the questions on sexual and emotional violence (n = 156, data not shown). When the association between having terminated a pregnancy and sexual violence alone and emotional violence alone are taken into account, the likelihood appears to be so few people reported a combination of sexual and emotional violence such that there was no apparent association with pregnancy outcomes.

Indirect abuse such as emotional violence was also related to pregnancy loss in this study. Psychological stress has been discussed as a possible factor for spontaneous abortion, both directly and indirectly through substance abuse such as smoking (*24-26*). In spite of this, few reports have examined links between emotional or psychological violence and miscarriage or stillbirth in comparison to those investigating the effects of physical or sexual violence. Measuring emotional violence is a difficult task, especially in a male-dominated culture where a husband's abuse or control

Table 3. Adjusted odds ratio (AOR) for the association between pregnancy termination and IPV according to NFHS-3

Type of violence	National (n = 59,674)
	Terminated pregnancy AOR (95% CI)
Violence	
No violence	1.00
Only physical	1.55 (1.42-1.68)***
Only sexual	1.63 (1.30-2.05)***
Only emotional	1.22 (1.00-1.49)*
Physical and sexual	2.23 (1.91-2.60)***
Physical and emotional	1.52 (1.35-1.70)***
Sexual and emotional	1.39 (0.79-2.44)
All types	2.07 (1.78-2.42)***
Any type of violence†	1.62 (1.51-1.73)***, [a]
Age	
15-24	1.00
25-34	1.60 (1.46-1.75)***
35-49	1.41 (1.27-1.57)***
Residence	
Urban	1.00
Rural	0.89 (0.82-0.97)**
Maternal education	
No education	1.00
Primary	1.14 (1.04-1.26)**
Secondary	1.04 (0.94-1.14)
Higher	0.94 (0.79-1.11)
Partner's education	
No education	1.00
Primary	1.06 (0.96-1.17)
Secondary	1.19 (1.08-1.31)***
Higher	1.46 (1.26-1.70)***
Other	1.28 (0.90-1.82)
Religion	
Hindu	1.00
Muslim	1.11 (0.99-1.26)
Other	0.82 (0.72-0.94)**
Standard of living index	
Low	1.00
Medium	1.00 (0.92-1.09)
High	1.06 (0.96-1.18)
Caste or tribe	
Scheduled caste	1.00
Scheduled tribe	0.77 (0.66-0.89)***
Other backward classes	1.09 (0.99-1.19)
Other	1.04 (0.94-1.15)
Parity	
0	1.00
1-2	1.20 (1.05-1.37)**
3-4	1.06 (0.92-1.22)
≥ 5	1.22 (1.04-1.43)*

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; † due to multicollinearity, this test was undertaken independently from other types of violence; [a] not associated with the living index.

of his wife is socially acceptable. Lack of a validated measure is a further complication. However, evidence from a study in Africa indicated that emotional violence was closely associated with recurrent fetal loss (*12*), which supports the current findings. Thus, the current study has provided valuable information on emotional violence and its association with reproductive health**.**

Maternal age and partner's education were significantly associated with termination of a pregnancy; and the risk of advanced age can be explained by its close association with spontaneous abortion and stillbirths (*27-29*), induced abortion in order to limit family size or spacing (*16*), or merely the time that passes with aging. That said, ambiguity in the classification of termination of a pregnancy in NFHS-3 hampered identification of reproductive outcomes related to the high educational level of a partner. Further discussion of socio-demographic characteristics is not possible due to the nature of NFHS-3 data.

A number of risk factors and determinants of IPV have been reported worldwide (*1-4,30-32*). The current findings were in accordance with well-known risk factors, such as better education for both men and women and being financially better off. Additionally, the relationship between higher parity and increased violence is presumed to exist because stresses related to having a number of children cause more partner abuse or because partner abuse could be a factor that leads to having a number of children (*1*). The social and economic issues relating to termination of a pregnancy in Indian culture must be studied further to elucidate new strategies to reduce the termination of pregnancies, especially among educated and wealthier women.

The current study had several limitations. First, the cross-sectional nature of this study made chronological assessment difficult, so the causal relationship between IPV and termination of a pregnancy could not be determined. Therefore, termination of a pregnancy might have induced violence by the husband. In addition, this analysis lacks a timeframe in relation to having experienced IPV and termination of a pregnancy. The survey asked about having experienced IPV and termination of a pregnancy without asking about exact dates so the causal relationship between IPV and termination of a pregnancy cannot be determined. The two possible scenarios are that a terminated pregnancy might have induced violence by the husband or that the violence occurred independently of pregnancy termination. Due to this limitation, this study found a clear association between IPV and termination of a pregnancy, but these events were only associated over the lifetime of the respondent. This needs to be considered when interpreting the current results, although the fact that they agree with the results of previous studies indicates that a causal relationship like that posited here is likely to exist.

Another limitation is underreporting and recall bias of having experienced violence and birth outcomes due to the sensitivity of the topic and retrospective nature of the survey. Even though the Cronbach's alpha for questions about violence was 0.82 and therefore questions asking about having experienced violence were considered internally consistent, some components of IPV that are specific to the Indian context might have been underestimated. Furthermore, insufficient categorization of types of terminated pregnancies in NFHS-3 prevented the collection of more precise data on individual associations between IPV and each type

of terminated pregnancy.

Recall bias could have dominated, especially as women distinguished the type of violence they had experienced. However, the domestic violence module adopted the Conflict Tactics Scale, which is oriented to behavior and avoids emotional or cognitive appraisal of violent behavior by asking about specific acts of violence such as "slapping" or "kicking" instead of having experienced violence in general. Moreover, interviewers were specifically trained to probe for answers with detailed information about experiences with IPV in a private and confidential setting to ensure that respondents were able to recall all IPV they had experienced. To ascertain pregnancy outcomes, each woman was asked about her reproductive history *via* a birth index so that complete birth histories could be obtained. To some extent, these efforts should eliminate variation and enhance recall by respondents.

Despite these limitations, a strength of this study is the fact that it utilized a wealth of large population-based data according to the NFHS, which covered all states in India. To the extent known, this is the first analysis of a population-based study dealing with partner violence and its relationship to pregnancy outcomes in India.

In conclusion, this study confirmed that Indian women who had been exposed to IPV were more likely to have terminated a pregnancy than those who were not. This finding reflects the delay in political and social support for maternal health in India. To protect women from violence and prevent unwarranted termination of a pregnancy, healthcare providers need to intervene by screening for and dealing with violence, and greater accessibility to health care and use of contraceptives are also needed. Empowering women by improving education and social support would also enhance their self-esteem and better equip them to take on challenging circumstances (*3*). Moreover, involvement of husbands, by education or counseling, is critical to reducing IPV against their wives as they are the perpetrators of violence.

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Antibody responses to lytic and latent human herpesvirus 8 antigens among HIV-infected patients in central China

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Summary **Human herpesvirus 8 (HHV8) is an important opportunistic infection of HIV/AIDS. However, very little is known about antibody seropositivities to HHV8 lytic and latent antigens among HIV-infected patients in China. Therefore, a cross-sectional study was conducted to explore HHV8 serostatus among 316 HIV-infected patients in a rural area of central China. The antibody seropositivity to HHV8 ORF65 (lytic) and LANA (latent) antigens was 12.7% and 10.4%, respectively. Patients who were naïve to antiretroviral therapy (ART) were more likely to be seropositive for antibodies to ORF65 (OR: 3.79; 95% CI: 1.71-8.42) and LANA (OR: 3.77; 95% CI: 1.55-9.14) than patients receiving ART. Patients having CD4+ cell counts less than 200 cells/mm^3 were more likely to be seropositive for LANA antibody (OR: 3.53; 95% CI: 1.44-8.64) and to have lower LANA antibody titer (p = 0.007). They were also more likely to be seropositive for ORF65 antibody (OR: 2.12; 95% CI: 0.94-4.78) and to have a lower ORF65 antibody titer (p = 0.065), though the difference was marginally significant. No associations between other viral coinfections studied and antibody seropositivity to either latent or lytic HHV8 antigens were identified. Study findings suggest that antibody responses to both lytic and latent HHV8 antigens among HIV patients in China were fairly high and were associated with immunodeficiency status and ART.**

Keywords: Antibody seropositivity, HHV8, HIV, LANA, ORF65

1. Introduction

Human herpesvirus 8 (HHV8), also known as Kaposi's sarcoma-associated herpesvirus (KSHV), has been linked to a number of clinical conditions, notably Kaposi's sarcoma (KS) (*1*), multicentric Castleman's disease (MCD) (*2*), and primary effusion lymphoma (PEL) (*3,4*). A higher incidence of HHV8 infection has been observed among HIV-infected patients. In fact, many epidemiological studies have suggested that there were concurrent epidemics of HHV8 and HIV commencing in the early 1980s (*5,6*). Due to HIV infection and the development of AIDS, individuals were more susceptible to opportunistic infections, including HHV8. Furthermore, progressive immunologic deterioration including CD4+ T-cell depletion and CD8+ T-cell dysfunction, the hallmarks of an untreated HIV infection, lead to impairment of immune control of HHV8 replication and therefore ultimately carcinogenesis, and possibly KS. Fortunately, the introduction of highly active antiretroviral therapy (HAART) in the past two decades has effectively led to a sharp decrease in the incidence of opportunistic infections and KS in HIV-infected patients in developed countries where such therapies were widely available (*7-9*).

HAART has been widely utilized throughout China since 2003 and has significantly reduced the mortality rate among HIV-infected patients despite the obstacle of drug resistance (*10-12*). As HIV-infected patients live longer *via* HAART, their chances for opportunistic infections, including HHV8 infection, might be enhanced as well. Given potentially shared transmission routes between HHV8 and other pathogens as well

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as the wide spectrum of pathogenic coinfections such as hepatitis B virus (HBV), hepatitis C virus (HCV), herpes simplex virus (HSV), and Epstein-Barr virus (EBV) among HIV-infected patients in China (*13*), their impact on HHV8 antibody response among HIV-infected patients should be ascertained.

HHV8 viral antigens are broadly categorized into two groups: lytic antigens (*e.g.* ORF65) and latent antigens (*e.g.* latent nuclear antigen or LANA) (*14*). Tests for antibodies to both lytic and latent HHV8 antigens can be used not only to identify HHV8 infection but also to understand their interactions with the host, *e.g.* the association between antibodies and HHV8 lytic and latent antigens and development of KS (*15,16*). Most previous studies on the seroprevalence of HHV8 infection report seropositivity of antibodies to any of these antigens without differentiating specific antibody responses to lytic and latent antigens. This could hamper thorough understanding of the HHV8 epidemic and host immune responses to HHV8 infection. As HIV-infected patients live longer when undergoing HARRT, they have a greater likelihood of developing KS. Identification of HHV8 serostatus and cofactors for KS development is paramount given the widespread use of HARRT.

Therefore, the current study specifically examined antibody seropositivities to lytic and latent HHV8 antigens among a sample of previously reported patients infected with HIV (*17*). Knowledge gained from this study should help to better understand host immune responses to HHV8 infection in the context of HIV infection and viral coinfections.

2. Materials and Methods

2.1. *Study sample*

As previously described (*17*), study participants were patients confirmed to be infected with HIV who had been registered with the National HIV/AIDS Information System and who were participating in an ongoing HIV cohort study that was established in 2006 in the City of Yuncheng, Shanxi Province in Central China. This site is where the HIV epidemic was first reported in 1996 and HIV was predominantly transmitted through plasma/blood donation or transfusion. Free antiretroviral treatment (ART) has been available for HIV-infected patients since 2003 in the area studied.

Venous blood was collected by trained nurses using disposable sterile needles and tubes and then transferred to a local laboratory within 4 h of collection. Serum samples were stored at −80°C for HHV8, HSV-1 and HSV-2, HBV, HCV, and EBV testing. Specimens were coded by unique identification numbers and were analyzed without knowledge of the individual identity of the study participant. This study was approved by the Institutional Review Board of Fudan University, China. All study participants provided written informed consent.

Data on participants' sociodemographic characteristics, HIV transmission mode, and receipt of HAART were obtained from the National HIV/AIDS Information System using a standard questionnaire form.

2.2. *HBV, HCV, HSV-1, HSV-2, and EBV testing*

HBV surface antigen (HBsAg) and anti-HCV IgG antibody were tested using an enzyme-linked immunosorbent assay (ELISA) (Wantai Biological Pharmacy Enterprise Co., Beijing, China). IgG antibodies to HSV-1 and HSV-2 were detected by type-specific ELISA (HerpeSelect 1 ELISA IgG Kit and HerpeSelect 2 ELISA IgG Kit, Focus Technologies, CA, USA). Anti-EBV nucleic antigen (EBNA) IgG antibody was tested for using ELISA (Euroimmun, Lübeck, Germany). All tests were performed by two independent technicians according to the manufacturers' standard protocols. Duplicate negative, positive, and blank controls were always used.

2.3. *HHV8 testing*

An immunofluorescence assay (IFA) was performed to detect the presence of lytic or latent antigen-specific antibodies, as previously reported (*18*). Briefly, *Spodoptera frugiperda* clone 9 cells infected with baculovirus expressing ORF65 antigen (lytic antigen) or ORF73 (latent nucleic antigen, LANA) were harvested, fixed, and spotted individually on separate slides for further sample testing. All serum samples were then tested at 1:40 dilution. Sera from KS patients who previously tested seropositive and healthy individuals who previously tested seronegative served as controls. Both lytic and latent antibody titers were further determined with IFA using serially diluted samples ranging from 1:40 to 1:10,240. Each slide was read independently by two experienced laboratory workers. Serostatus was categorized as antibody seropositivity for lytic antigen (ORF65), latent antigen (LANA), and ANY and BOTH lytic and latent antigens as previously reported (*19*).

2.4. *CD4+ and CD8 T+ cell counts and HIV RNA quantification*

The absolute number of CD4+ and CD8+ T lymphocytes in peripheral blood was estimated using a fluorescence-activated cell analyzer with monoclonal antibodies (BD FACSCount System, BD Biosciences, San Jose, CA, USA). Plasma HIV viral loads were quantified using the Amplicor HIV-1 RNA Monitor Test v1.5 (Roche Diagnostics Alameda, CA, USA).

2.5. *Statistical analysis*

Original questionnaires and laboratory testing results were entered and managed in EpiData3.0, and then the database was transferred to an SPSS database for further management and analysis. Seroprevalence of both lytic and latent antibodies was calculated. Pearson's Chi-squared tests were performed to evaluate differences in seroprevalence between subgroups. Nonparametric tests (Mann-Whitney *U* tests) were used to assess the difference in geometric mean titers (GMTs) of antibodies between different groups. Concordance between the latent and lytic serology assay was assessed using the kappa statistic. Univariate logistic regression analysis was first done and then followed by multivariate analysis to explore associations with seropositivity for both lytic and latent antibodies. Odds ratios (OR) and their 95% confidence intervals (95% CI) were calculated and used to determine whether a variable was associated with antibodies against LANA, ORF65, ANY, or BOTH. All statistical analyses were performed using SPSS software 15.0 (SPSS, Chicago, Illinois, USA) and GraphPad Prism 5.0 (GraphPad, La, Jolla, CA, USA). A two-sided *p*-value of 0.05 or less was considered statistically significant.

3. Results

3.1. *Sociodemographic characteristics and seroprevalence of viral coinfections*

A total of 316 HIV-infected patients were included in this study. Their sociodemographic characteristics are shown in Table 1. Of the sample, 53.8% were males

Table 1. Socio-demographic characteristics of study participants

Item	Male (170) *n* (%)	Female (146) *n* (%)	Total (316) *n* (%)
Ethnicity ($p = 0.252$)			
Han	167 (98.2)	146 (100.0)	313 (99.1)
Other	3 (1.8)	0 (0)	3 (0.9)
Age group ($p = 0.530$)			
0-18	5 (2.9)	6 (4.1)	11 (3.5)
19-49	123 (72.4)	111 (76.0)	234 (74.1)
50+	42 (24.7)	29 (19.9)	71 (22.5)
Marital status ($p = 0.346$)			
Married	156 (91.8)	136 (93.2)	292 (92.4)
Single	7 (4.1)	8 (5.5)	15 (4.7)
Divorced/widowed	7 (4.1)	2 (1.4)	9 (2.8)
Education ($p < 0.001$)			
Illiterate	10 (5.9)	17 (11.6)	27 (8.5)
Primary school	52 (30.6)	67 (45.9)	119 (37.7)
Middle school	95 (55.9)	59 (40.4)	154 (48.7)
High school or higher	13 (7.6)	3 (2.1)	16 (5.1)
Farmer ($p = 0.409$)			
Yes	154 (90.6)	136 (93.2)	290 (91.8)
No	16 (9.4)	10 (6.8)	26 (8.2)
Monthly income (RMB, $p = 0.779$)			
< 1,000	97 (57.1)	82 (56.2)	179 (55.6)
1,001-2,000	35 (20.6)	36 (24.7)	71 (22.5)
2,001-3,000	12 (7.1)	10 (6.8)	22 (7.0)
> 3,000	26 (15.3)	18 (12.3)	44 (13.9)
Alcohol Consumption ($p < 0.001$)			
Yes	26 (15.3)	3 (2.1)	29 (9.2)
No	144 (84.7)	143 (97.9)	287 (90.8)
Smoking ($p < 0.001$)			
Yes	109 (64.1)	5 (3.4)	114 (36.4)
No	61 (35.9)	141 (96.6)	202 (63.6)
HBsAg ($p = 0.786$)			
Positive	13 (7.6)	10 (6.8)	23 (7.3)
Negative	157 (92.4)	136 (93.2)	293 (92.7)
HCV ($p < 0.001$)			
Positive	142 (83.5)	93 (63.7)	235 (74.4)
Negative	28 (16.5)	53 (39.3)	81 (25.6)
EBV ($p = 0.096$)			
Positive	161 (94.7)	131 (89.7)	292 (92.4)
Negative	9 (5.3)	15 (10.3)	24 (7.6)
HSV-1 ($p = 0.430$)			
Positive	130 (76.5)	106 (72.6)	236 (74.7)
Negative	40 (23.5)	40 (27.4)	80 (25.3)
HSV-2 ($p = 0.012$)			
Positive	26 (15.3)	39 (26.7)	65 (20.6)
Negative	144 (84.7)	107 (73.3)	251 (79.4)

and 46.2% were females with a mean age of 42.03 years (S.D. = 10.25). About 99.1% of the participants belonged to the Han ethnic group. Males were more likely to drink alcohol ($p < 0.001$) and smoke ($p < 0.001$) than females. No gender differences existed for other demographic characteristics (Table 1).

Among the participants, 23 (7.3%) were seropositive for HBsAg, 235 (74.4%) for HCV, 236 (74.4%) for HSV-1, 65 (20.6%) for HSV-2, and 292 (92.4%) for EBV. Males had a higher prevalence of HCV infection but lower prevalence of HSV-2 infection than females (Table 1).

3.2. *Prevalence and correlates of HHV8 lytic and latent antibody seropositivity*

The serostatus of lytic and latent antibodies were determined separately. Antibody seropositivity was 12.7% for lytic antigen (ORF65) and 10.4% for latent antigen (LANA). The two serology assays showed a moderate concordance (Kappa = 0.582). Two separate multiple logistic regression analyses were performed to explore independent correlates with ORF65 and LANA antibody seropositivity by adjusting for gender and age group. As shown in Table 2, both ORF65 and LANA antibody seropositivity were significantly associated with ART and CD4+ cell counts. Participants who were ART-naïve were more likely to be positive for ORF65 antibody (OR: 3.79; 95% CI: 1.71-8.42) and LANA antibody (OR: 3.77; 95% CI: 1.55-9.14) than those receiving ART. Patients having CD4+ cell counts less than 200 cells/mm^3 were more likely to be seropositive for LANA antibody (OR: 3.53; 95% CI: 1.44-8.64) and to have a lower LANA antibody titer ($p = 0.007$). They were also more likely to be seropositive for ORF65 antibody (OR: 2.12; 95% CI: 0.94-4.78) and to have

Table 2. Multivariate analysis of correlates with HHV8 lytic and latent antibody seropositivities among study participants

Characteristics/Risk factor	LANA			ORF65		
	Positives/total (%)	OR (95%CI)*	*p*-value*	Positives/total (%)	OR (95% CI)*	*p*-value*
Socio-demographics						
Gender						
Male	11/170 (6.5)	1.00		18/170 (10.6)	1.00	
Female	22/146 (15.1)	2.53 (1.12-5.73)	0.026*	22/146 (15.1)	1.38 (0.67-2.85)	0.385
Age group						
0-18	2/11 (18.2)	1.00		3/11 (27.3)	1.00	
19-49	24/243 (10.3)	0.36 (0.06-2.16)	0.265	30/234 (12.8)	0.33 (0.07-1.60)	0.172
50 +	7/71 (9.9)	0.38 (0.05-2.74)	0.340	7/71 (9.9)	0.28 (0.05-1.65)	0.162
HIV-related factors						
ART						
Yes	19/238 (8.0)	1.00		22/238 (9.2)	1.00	
No	14/78 (17.9)	3.77 (1.55-9.14)	0.003**	18/78 (23.1)	3.79 (1.71-8.42)	0.001**
CD4 (cell/mm^3)						
> 200	15/187 (8.0)	1.00		22/187 (11.8)	1.00	
≤ 200	18/129 (14.0)	3.53 (1.44-8.64)	0.006**	18/129 (14.0)	2.12 (0.94-4.78)	0.07
CD8 (cell/mm^3)						
> 400	27/251 (10.8)	1.00		32/251 (12.7)	1.00	
≤ 400	6/65 (9.2)	0.73 (0.25-2.14)	0.571	8/65 (12.3)	0.97 (0.37-2.57)	0.955
Viral load (copies/mL)						
≤ 400	4/25 (16.0)	1.00		6/25 (24.0)	1.00	
> 400	29/291 (10.0)	0.47 (0.12-1.86)	0.287	34/291 (11.7)	0.42 (0.13-1.35)	0.143
Duration of HIV infection (yr)						
≤ 10	14/97 (14.4)	1.00		18/97 (18.6)	1.00	
> 10	19/219 (8.7)	0.59 (0.25-1.38)	0.220	22/219 (10.0)	0.54 (0.25-1.19)	0.129
Co-infections						
HBsAg						
Negative	29/293 (9.9)	1.00		35/293 (11.9)	1.00	
Positive	4/23 (17.4)	2.36 (0.67-8.47)	0.182	5/23 (21.7)	2.41 (0.75-7.72)	0.140
HCV						
Negative	10/81 (12.3)	1.00		13/81 (16.0)	1.00	
Positive	23/235 (9.8)	0.52 (0.89-1.44)	0.211	27/235 (11.5)	0.63 (0.25-1.58)	0.323
EBV						
Negative	2/24 (8.3)	1.00		4/24 (16.7)	1.00	
Positive	31/292 (10.6)	1.88 (0.36-9.72)	0.452	36/292 (12.3)	0.93 (0.27-3.25)	0.909
HSV-1						
Negative	8/80 (10.0)	1.00		9/80 (11.3)	1.00	
Positive	25/236 (10.6)	1.30 (0.52-3.24)	0.574	31/236 (13.1)	1.42 (0.60-3.36)	0.427
HSV-2						
Negative	26/251 (10.4)	1.00		32/251 (12.7)	1.00	
Positive	7/65 (10.8)	1.03 (0.39-2.73)	0.956	8/65 (12.3)	0.95 (0.38-2.37)	0.917

*The odds ratio (OR) and 95% CI were obtained by adjusting for other variables listed in the table.

a lower ORF65 antibody titer (p = 0.065) although these associations were marginally significant. No associations between other examined viral coinfections and antibody seropositivity to either latent or lytic HHV8 antigen were identified.

3.3. *Correlates of antibody seropositivity for ANY and BOTH lytic and latent HHV8 antigens*

The seropositivity of antibodies to ANY and BOTH lytic and latent HHV8 antigens was 15.8% and 7.3%, respectively. Regression analyses revealed that patients who were ART-naïve (OR: 3.67; 95% CI: 1.75-7.69, p = 0.001) or had low CD4+ cell counts (OR: 2.71; 95% CI: 1.29-5.68, p = 0.008) were more likely to be antibody seropositive for ANY lytic and latent HHV8 antigens (Table 3). They were also more likely to be antibody seropositive for BOTH lytic and latent HHV8 antigens (Table 3).

3.4. *Antibody titers by different characteristics*

Geometric mean titers (GMT) of antibodies to lytic and latent antigens of HHV8 were compared according to the patient's ART status, CD4+ count, CD8+ count, and duration of HIV infection. Patients with CD4+ T cell counts less than 200 cell/mm^3 had lower antibody titers for latent antigen (p = 0.007) and lytic antigens (p = 0.065) (Figure 1). Antibody titers to lytic and latent antigens of HHV8 did not differ significantly with the patient's ART status, CD8+ count, or duration of HIV infection (Figure 1).

Table 3. Multivariate analysis of correlates with ANY and BOTH HHV8 lytic and latent antibody seropositivity among study participants

Characteristics/Risk factor	ANY			BOTH		
	Positives/total (%)	OR (95% CI)	p-value	Positives/total (%)	OR (95% CI)	p-value*
Socio-demographics						
Gender						
Male	22/170 (12.9)	1.00		7/170 (4.7)	1.00	
Female	28/146 (19.2)	1.51 (0.78-2.91)	0.225	16/146 (11.0)	2.83 (1.05-7.62)	0.039*
Age group						
0-18	3/11 (27.3)	1.00		2/11 (18.2)	1.00	
19-49	38/234 (16.2)	0.39 (0.08-1.79)	0.229	16/234 (6.8)	0.26 (0.04-1.69)	0.185
50 +	9/71 (12.7)	0.32 (0.06-1.75)	0.191	5/71 (7.0)	0.31 (0.03-2.59)	0.181
HIV-related factors						
ART						
Yes	29/238 (12.2)	1.00		12/238 (5.0)	1.00	
No	21/78 (25.9)	3.67 (1.75-7.69)	0.001**	11/78 (14.1)	4.36 (1.55-12.27)	0.005*
CD4 (cell/mm^3)						
> 200	25/187 (13.4)	1.00		12/187 (6.4)	1.00	
≤ 200	25/129 (19.4)	2.71 (1.29-5.68)	0.008**	11/129 (8.5)	2.82 (0.98-8.13)	0.054*
CD8 (cell/mm^3)						
> 400	40/251 (15.9)	1.00		19/251 (7.6)	1.00	
≤ 400	10/65 (15.4)	0.87 (0.36-2.08)	0.748	4/65 (6.2)	0.78 (0.21-2.91)	0.718
Viral load (copies/mL)						
≤ 400	6/25 (24.0)	1.00		4/25 (16.0)	1.00	
> 400	44/291 (15.1)	0.51 (0.16-1.61)	0.252	19/291 (6.5)	0.31 (0.07-1.35)	0.120
Duration of HIV infection (yr)						
≤ 10	20/97 (20.6)	1.00		12/97 (12.4)	1.00	
> 10	30/219 (13.7)	0.65 (0.32-1.33)	0.240	11/219 (5.0)	0.41 (0.15-1.12)	0.077
Co-infections						
HBsAg						
Negative	44/239 (15.0)	1.00		20/293 (6.8)	1.00	
Positive	6/23 (26.1)	2.42 (0.82-7.12)	0.108	3/23 (13.0)	2.30 (0.53-9.98)	0.265
HCV						
Negative	15/81 (18.5)	1.00		8/81 (9.9)	1.00	
Positive	35/235 (14.9)	0.62 (0.26-1.42)	0.260	15/235 (6.4)	0.48 (0.14-1.61)	0.231
EBV						
Negative	4/24 (16.7)	1.00		2/24 (8.3)	1.00	
Positive	46/292 (15.8)	0.82 (0.24-2.79)	0.752	21/292 (7.2)	0.74 (0.14-4.11)	0.736
HSV-1						
Negative	13/80 (16.3)	1.00		4/80 (5.0)	1.00	
Positive	37/236 (15.7)	0.87 (0.41-1.84)	0.716	19/236 (8.1)	2.23 (0.64-7.70)	0.205
HSV-2						
Negative	39/251 (15.5)	1.00		19/251 (7.6)	1.00	
Positive	11/65 (16.9)	1.08 (0.48-2.43)	0.839	4/65 (6.2)	0.77 (0.25-2.66)	0.684

*The odds ratio (OR) and 95% CI were obtained by adjusting for other variables listed in the table.

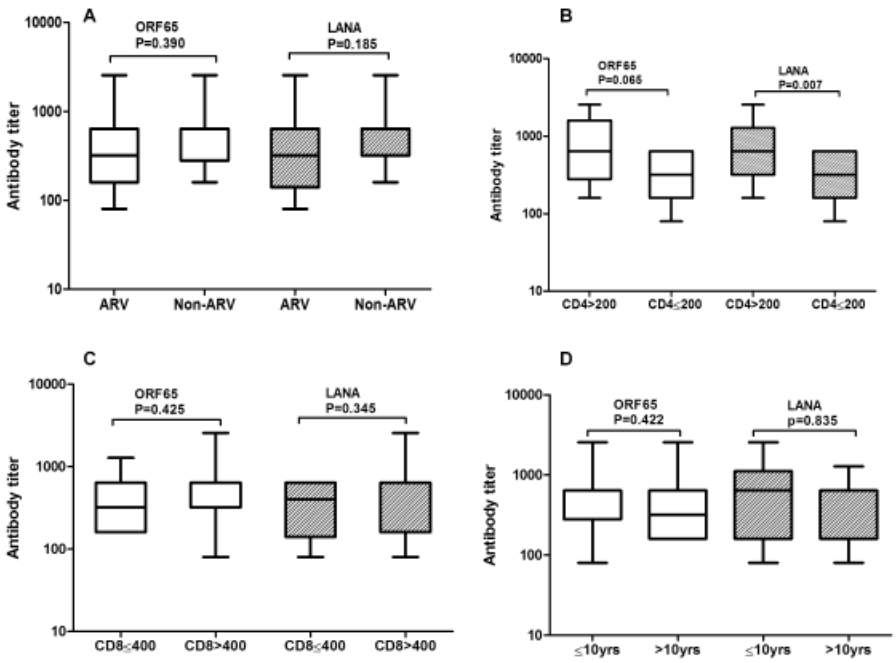

Figure 1. Box-and-whisker plots of HHV8 lytic and latent antibody titer by HIV-related factors. (A) Antibody titer for patients receiving ART and not receiving ART. **(B)** Antibody titer for patients with CD4 ≤ 200 cells/mm^3 and CD4 > 200 cells/mm^3. **(C)** Antibody titer for patients with CD8 ≤ 400 cells/mm^3 and CD8 > 400 cells/mm^3. **(D)** Antibody titer for patients infected with HIV ≤ 10 yr and those infected with HIV > 10 yr. * Titers for different groups were calculated and compared using Mann-Whitney *U*. "yr" stands for years.

4. Discussion

The present study is the first to extensively examine seroprevalence and epidemiologic characteristics of antibodies to lytic and latent HHV8 antigens among Chinese HIV-infected patients. This study found a seroprevalence of 12.7% for antibodies to lytic antigen (ORF65), 10.4% for antibodies to latent antigen (LANA), 15.8% for antibodies to ANY of the two antigens, and 7.3% for antibodies to BOTH antigens. Previous studies from China often reported only an overall seroprevalence of antibodies to ANY lytic and latent antigens ranging from 16.3% to 43.2% (*20-23*), and higher than that (15.8%) in the present study. This further suggests that HHV8 infection is unevenly distributed across populations and geographical regions in China (*24*). Moreover, the present study, by showing individualized antibody responses to the two antigens, facilitates a better understanding of host immune responses to HHV8 infection.

Since the introduction of ART, a dramatic decline in the incidence of HIV/AIDS opportunistic infections has been witnessed worldwide (*25,26*). Data have shown that ART may also influence HHV8 infection among HIV-infected patients in a number of ways and decrease the risk of AIDS-KS (*16,27,28*). In the present study, both ART and CD4+ T cell counts were significantly correlated with HHV8 seropositivity, further suggesting the impact of ART on host immune responses to HHV8 infection.

In this study, HIV-infected patients who received ART treatment were less likely to be positive for both lytic (ORF65) and latent (LANA) antibodies, although the antibody titers of patients receiving ART and not receiving ART did not differ significantly. Since LANA is one of the few viral proteins expressed during latency and is one of the most immunogenetic HHV8 antigens (*14*), detection of antibodies to LANA is primarily used as a marker of an established persistent latent HHV8 infection. The current findings, consistent with results from the Swiss HIV Cohort Study (*16*), suggest that ART could have a positive effect on HHV8 infection. Nevertheless, whether or not ART can alter the latency of HHV8 infection remains an important scientific question that warrants further study in the near future.

CD4+ and CD8+ T cells play important roles in protection against intracellular pathogens including HHV8. In the present study, HIV-infected patients with CD4+ cell counts less than 200 cell/mm^3 were consistently found to have a higher seroprevalence but a lower titer of both lytic (ORF65) and latent (LANA) antibodies. Previous studies also found that HIV-infected patients with CD4+ cell counts less than 200 cell/mm^3 had a higher seroprevalence of lytic (ORF65) antibody (*15,19*). However, they found no association between CD4+ cell counts and seroprevalence of latent (LANA) antibody (*15,19*). A possible explanation for this discrepancy is that the current study included both patients who were ART-naïve and those receiving ART whereas the two cited studies included either only ART-

naïve patients (*15*) or only patients receiving ART but not both. That said, the CD8+ cell count was not significantly correlated with antibody responses to any of the lytic and latent antigens. This is most likely due to the fact that CD8+ T-cells are mostly involved in cellular responses but not humeral responses.

In addition to HHV8 coinfection, HIV patients are also living with other pathogenic viral coinfections due to shared transmission modes (*13*). The current study found a high prevalence of coinfections with HBV, HCV, HSV-1, HSV-2, and EBV. However, none were found to be significantly associated with either of the lytic and latent HHV8 antibodies. This finding is consistent with that of a study conducted among a sample of HIV patients in the United States (*19*) but is inconsistent with that of a study conducted among a sample of Chinese patients with chronic hepatitis B (*20*). More intensive and extensive research is urgently needed to address such questions.

This study had a couple of limitations. First, the capacity to make valid causal inferences might be limited due to the nature of a cross-sectional study design. Second, none of the study participants had KS. Therefore, the potential relationship between host antibody responses to lytic and latent HHV8 antigens and KS risk has by no means been defined.

In conclusion, HIV patients in Central China had relatively high antibody seropositivity to lytic and latent HHV8 antigens, and this seropositivity was significantly associated with ART status and CD4+ cell counts. More extensive and prospective studies are urgently needed to address controversial findings and to better understand interactions between HHV8 and the host in the context of HIV-induced immunodeficiency being treated by or not being treated by ART.

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Original Article

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Platelet-derived growth factor receptor kinase inhibitor AG-1295 promotes osteoblast differentiation in MC3T3-E1 cells *via* the Erk pathway

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Summary **Previous studies have conflicting views on the effect of platelet-derived growth factor (PDGF)/PDGF receptor (PDGFR) signaling on osteogenesis. The current study investigated the effect of PDGF receptor-beta (PDGFR-β) inhibition by AG-1295 on the osteogenic differentiation of the mouse pre-osteoblastic cell line MC3T3-E1. Osteogenic differentiation was induced by treatment with β-glycerophosphate, ascorbic acid, and dexamethasone along with or absent AG-1295. Results showed that AG-1295 significantly increased alkaline phosphatase (ALP) activity and enhanced the formation of mineralized nodules in a dose-dependent manner. Furthermore, treatment with AG-1295 resulted in up-regulated mRNA expression of the osteogenic marker genes collagen type I (*Col1A*), runt-related transcription factor 2 (*Runx2*), osterix (*Osx*), tissue-nonspecific alkaline phosphatase (*Tnap*), and osteocalcin (*Ocn*). Consistent with its effect on osteoblast differentiation, AG-1295 also significantly suppressed the phosphorylation of Erk1/2 in MC3T3-E1 cells. In conclusion, findings suggest that blocking the PDGFR-β pathway with AG1295 markedly promotes osteoblast differentiation and matrix mineralization in mouse osteoblastic MC3T3-E1 cells and that the Erk1/2 pathway might participate in this process.**

Keywords: Platelet-derived growth factor receptor-beta, AG-1295, extracellular signal-regulated kinases 1 and 2, matrix mineralization

1. Introduction

Platelet-derived growth factor receptors (PDGFR) are cell surface tyrosine kinase receptors for members of the PDGF family (*1*). PDGF/PDGFR signaling is reported to be involved in the regulation of various cell functions by activating three major signal transduction pathways including MAPK/Erk, PI3K, and PLC-γ (*2-5*).

Accumulating evidence suggests that PDGFR signaling plays an important role in the regulation of osteoblasts or mesenchymal stem cells (MSCs). Recent experiments suggest that activation of PDGFR signaling by PDGF factor BB plays a positive role in bone formation (*6,7*). Fierro *et al.* (*8*) showed that inhibition of PDGFR activity by imatinib *in vitro* partially suppressed osteogenesis of MSCs. That said, some literature has suggested that PDGFR signaling suppresses osteoblast differentiation (*9-11*). For example, Tokunaga *et al.* (*12*) found that knockout of the PDGFR-β gene in murine MSCs enhanced osteogenic differentiation. Clinically, long-term inhibition of PDGFR signaling by imatinib therapy has been reported to promote bone formation in patients with chronic myeloid leukemia (CML) (*11,13*). In particular, a recent study indicated that PDGFR signaling inhibition by AG-1296 does not significantly contribute to the osteogenic differentiation of MSC cells, as indicated by alkaline phosphatase (ALP)

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activity, osteogenic marker gene expression, and matrix calcium deposition staining (*14*).

Some previous studies, however, have reported that PDGFR signaling inhibits osteogenesis. Furthermore, the mechanisms by which PDGFR signaling inhibits osteogenic differentiation remain unclear. Therefore, the present study examined the effect of PDGFR-β inhibition by Tyrphostin AG-1295, a potent PDGFR-β blocker (*15*) on matrix mineralization in MC3T3-E1 cells. Findings suggest that blocking of PDGFR signaling by Tyrphostin AG-1295 promotes osteoblast differentiation and mineralization and that activation of the Erk1/2 pathway might be involved in this process.

2. Materials and Methods

2.1. *Chemicals and antibodies*

PDGFR inhibitor AG-1295 was purchased from Calbiochem (San Diego, CA, USA). Alpha-modified minimum essential medium (α-MEM), Dulbecco's modified Eagle's medium (DMEM), and Fetal bovine serum (FBS) were purchased from Gibco (Rockville, MD, USA). β-Glycerophosphate, ascorbic acid, and dexamethasone were purchased from Sigma (St. Louis, MO, USA). Rabbit monoclonal antibodies for Erk1/2 and p-Erk1/2 were obtained from Cell Signaling Technology (Beverly, MA, USA).

2.2. *Cells and osteoblast differentiation induction*

The osteoblastic cell line MC3T3-E1 (subclone 14), which was established from normal mouse calvaria (*16*), was purchased from the Cell Bank of the Type Culture Collection of the Chinese Academy of Sciences. MC3T3-E1 cells were cultured in α-MEM supplemented with 10% FBS and 1% penicillin-streptomycin. To stimulate mineralization, MC3T3-E1 cells at 1×10^4 cells/well were first cultured in 24 or 6-well plates to 80% to 90% confluence, and then cells were placed in osteogenic media (DMEM with 10% FBS, 1% penicillin-streptomycin, 10 mmol/L β-glycerophosphate, 50 μg/mL ascorbic acid, and 10 nmol/L dexamethasone) in the absence (the positive control group) or presence of AG-1295 (1, 10, and 20 μmol/L) for the times indicated. The negative control group was placed in basic medium (DMEM with 10% FBS, 1% penicillin-streptomycin) alone.

2.3. *ALP activity*

MC3T3-E1 cells were cultured in 24-well (1×10^4 cells/well) plates during osteogenic induction as described above. After treatment, cell lysates were prepared with 100 μL assay buffer containing 25 mM Tris-HCl (pH 7.4) and 0.5% Triton X-100. Fifty μL of each sample was mixed with 100 μL *p*-nitrophenyl phosphate (PNPP) substrate (Sigma) and cells were incubated at 37°C for 30 min. The absorbance of the colored product was detected at 405 nm. Cellular ALP activity was normalized to total protein content using the bicin-choninic acid (BCA) method.

2.4. *Analysis of mineralization*

After osteogenic induction, mineral deposition was assessed by staining with Alizarin-Red on days 21, 25, and 30. The cells were fixed in formalin for 20 min at room temperature and washed with distilled water. A 2% Alizarin Red solution was added to the fixed cells and cells were incubated for 10-20 min. Culture plates were rinsing with distilled water and then imaged.

2.5. *Quantitative real-time polymerase chain reaction (RT-qPCR)*

Total RNA was prepared at the indicated time points using Total RNA Kit I (Omega, Bio-Tek, Norcross, GA, USA) in accordance with the manufacturer's instructions. Complementary DNA (cDNA) was synthesized using ReverTra Ace qPCR RT Kit (Toyobo). Real-time PCR was performed as usual with SYBR Green mix (Toyobo, Osaka, Japan) and a Lightcycler 480 (Roche Applied Science, Mannheim, Germany). Gene-specific primer sequences are listed in Table 1. Gene expression was normalized with the acidic ribosomal phosphoprotein P0 (*36B4*) of the mouse housekeeping gene (*17*). Cycling conditions were 94° C, 15 min, followed by 40 cycles of 94°C, 15 sec; 57°C, 20 sec; and 72°C, 10 sec.

2.6. *Western blotting*

After treatment, cells were lysed in lysis buffer (50 mmol/L Tris-HCl, pH 7.4, 150 mmol/L NaCl, 1 mmol/L ethylenediaminetetraacetic acid, 0.25% Na-deoxycholate, 1% NP-40, 1 mmol/L phenylmethane-sulfonylfluoride, 1 mmol/L sodium orthovanadate, 1 μg/mL leupeptin, 1 μg/mL aprotinin, and 1 μg/mL pepstatin). Equivalent amounts (25 μg) of protein were separated on 12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) gels and subsequently transferred to Hybond-enhanced chemiluminescence (ECL) nitrocellulose membranes. Membranes were probed with antibodies against total Erk1/2, phospho- Erk1/2, and Actin. Protein bands were observed using ECL and specific bands were detected with X-film.

2.7. *Statistical analysis*

Statistically significant differences between groups were determined using an unpaired Student's *t*-test. Statistical significance was defined as $p < 0.05$.

3. Results

3.1. *PDGFR-β kinase inhibitor Tyrphostin AG1295 increased ALP activity during the early stages of osteoblast differentiation in MC3T3-E1 cells*

ALP activity was assessed as an early indicator of osteoblastic lineage to study the effect of AG-1295 on the osteogenic differentiation of MC3T3-E1 cells. ALP activity was determined on days 3, 6, 9, and 14 with osteogenic induction in the presence or absence of AG-1295 (1, 10, and 20 μmol/L). Figure 1 shows the ALP activity of MC3T3-E1 cells under different conditions. ALP activity in all groups of MC3T3-E1 cells peaked on day 9 and decreased on day 14, which is consistent with the results of previous studies. ALP activity increased significantly by 20 μmol/L AG-1295 on days 3, 6, and 9 ($p < 0.05$). However, there were no significant differences in ALP activity in the groups on day 14. This indicates that AG-1295 increased ALP activity during the early stages of osteoblast differentiation in MC3T3-E1 cells.

3.2. *PDGFR-β kinase inhibitor Tyrphostin AG-1295 enhanced matrix mineralization in MC3T3-E1 cells*

MC3T3-E1 cells were stained with Alizarin Red to detect nodule mineralization on days 21, 25, and 30. As shown in Figure 2, AG-1295 markedly enhanced nodule mineralization in a concentration-dependent manner. Cells treated with 10 μmol/L and 20 μmol/L AG-1295 had obvious mineralized nodules on day 21, while MC3T3-E1 cells cultured in osteogenic media had obvious mineralized nodules prior to day 30 (Figure 2).

3.3. *Effect of AG-1295 on the expression of osteoblast-specific marker genes in MC3T3-E1 cells during osteoblast induction*

Real-time PCR results for osteogenic marker genes collagen type I (*Col1A*), runt-related transcription factor 2 (*Runx2*), osterix (*Osx*), tissue-nonspecific alkaline phosphatase (*Tnap*), osteocalcin (*Ocn*), and progressive ankylosis (*AnK*) are shown in Figure 3. According to the measured level of ALP activity and Alizarin Red S staining, blocking PDGFR-β with AG-1295 could elevate the mRNA expression of most of the osteogenic markers investigated. After AG-1295 treatment, significantly up-regulated mRNA levels of *Col1A*, *Runx2*, *Osx*, *Tnap*, and *Ocn* were identified on days 9 and 14. However, AG-1295 down-regulated the expression of *Ank*.

3.4. *Effect of PDGF-β inhibition by AG-1295 on the Erk1/2 pathway in MC3T3-E1 cells*

To evaluate whether the Erk1/2 pathway is involved in the regulation of AG-1295 as part of mineralization in

Table 1. Primer pairs used for quantitative real-time reverse transcription-polymerase chain reaction

Gene	Forward (5' to 3')	Reverse (5' to 3')	Reference
Col1A	CACCCCAGCCGCAAAGAGT	CGGGCAGAAAGCACAGCACT	(*23*)
Tnap	GGGGACATGCAGTATGAGTT	GGCCTGGTAGTTGTTGTGAG	(*23*)
Runx2	CTCAGTGATTTAGGGCGCATT	AGGGGTAAGACTGGTCATAGG	(*24*)
Ocn	TGCTTGTGACGAGCTATCAG	GAGGACAGGGAGGATCAAGT	(*24*)
Osx	GGAGGTTTCACTCCATTCCA	TAGAAGGAGCAAGGGGACAGA	(*25*)
Ank	GAACTATCTGCCGCAC	AGGCGAGTAAACGCAA	(*23*)
36B4	AAGCGCGTCCTGGCATTGTCT	CCGCAGGGGCAGCAGTGGT	(*24*)

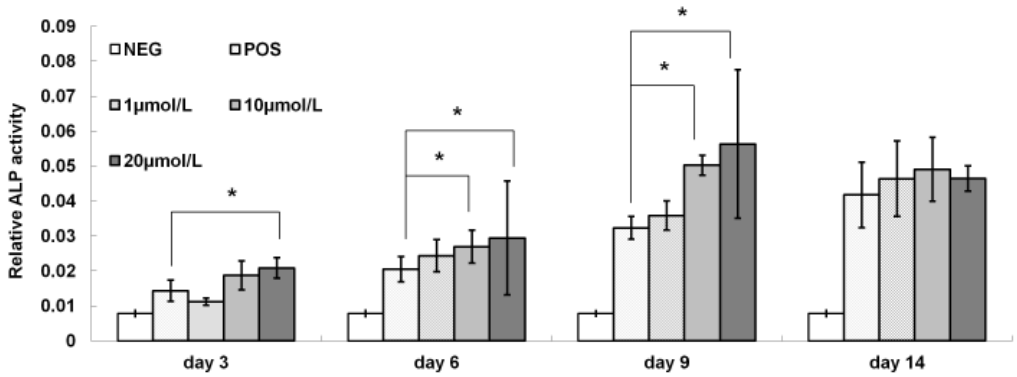

Figure 1. The effect of Tyrphostin AG1295 treatment on alkaline phosphatase (ALP) activity in MC3T3-E1 cells. Cells were incubated in osteogenic media with 0.1% DMSO (POS) and with 1, 10, and 20 μmol/L of Tyrphostin AG1295. ALP activity was determined on days 3, 6, 9, and 14. Cells cultured in basic medium containing 0.1% DMSO alone served as a negative control (NEG). Values are expressed as the mean ± S.D. ($n = 4$ per each group). Asterisks indicate statistically significant differences between groups (* $p < 0.05$).

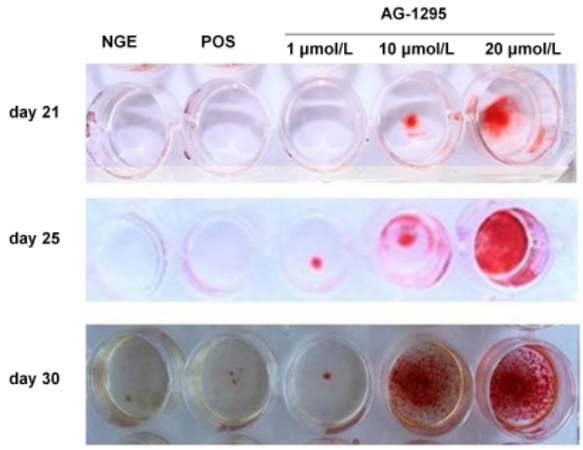

Figure 2. The effect of Tyrphostin AG1295 treatment on the formation of mineralized nodules in MC3T3-E1 cells. Cells were cultured in osteogenic media for 21, 25, and 30 days in the presence of 0.1% DMSO (POS) or 20 μmol/L Tyrphostin AG1295 and subjected to alizarin red staining. For the negative control (NEG), MC3T3-E1 cells were cultured in basic medium containing 0.1% DMSO alone.

MC3T3-E1, the expression of total and phosphorylated Erk1/2 protein was detected with Western blotting. As shown in Figure 4, a marked level of phosphorylated Erk1/2 protein was detected on 10 day of osteogenic induction, while no significant changes in the level of total Erk1/2 were noted. Furthermore, the phosphorylation activation of Erk1/2 was significantly suppressed by 20 μmol/L Tyrphostin AG-1295 in MC3T3-E1 cells cultured in osteogenic media on day 10.

4. Discussion

The current study demonstrated that blocking PDGFR signaling with Tyrphostin AG-1295 significantly promoted osteoblast mineralization. AG-1295 increased ALP activity in the early stages of osteoblast differentiation in MC3T3-E1 cells and enhanced matrix mineralization in the later stages of osteoblast differentiation. Like osteoblast phenotype induction,

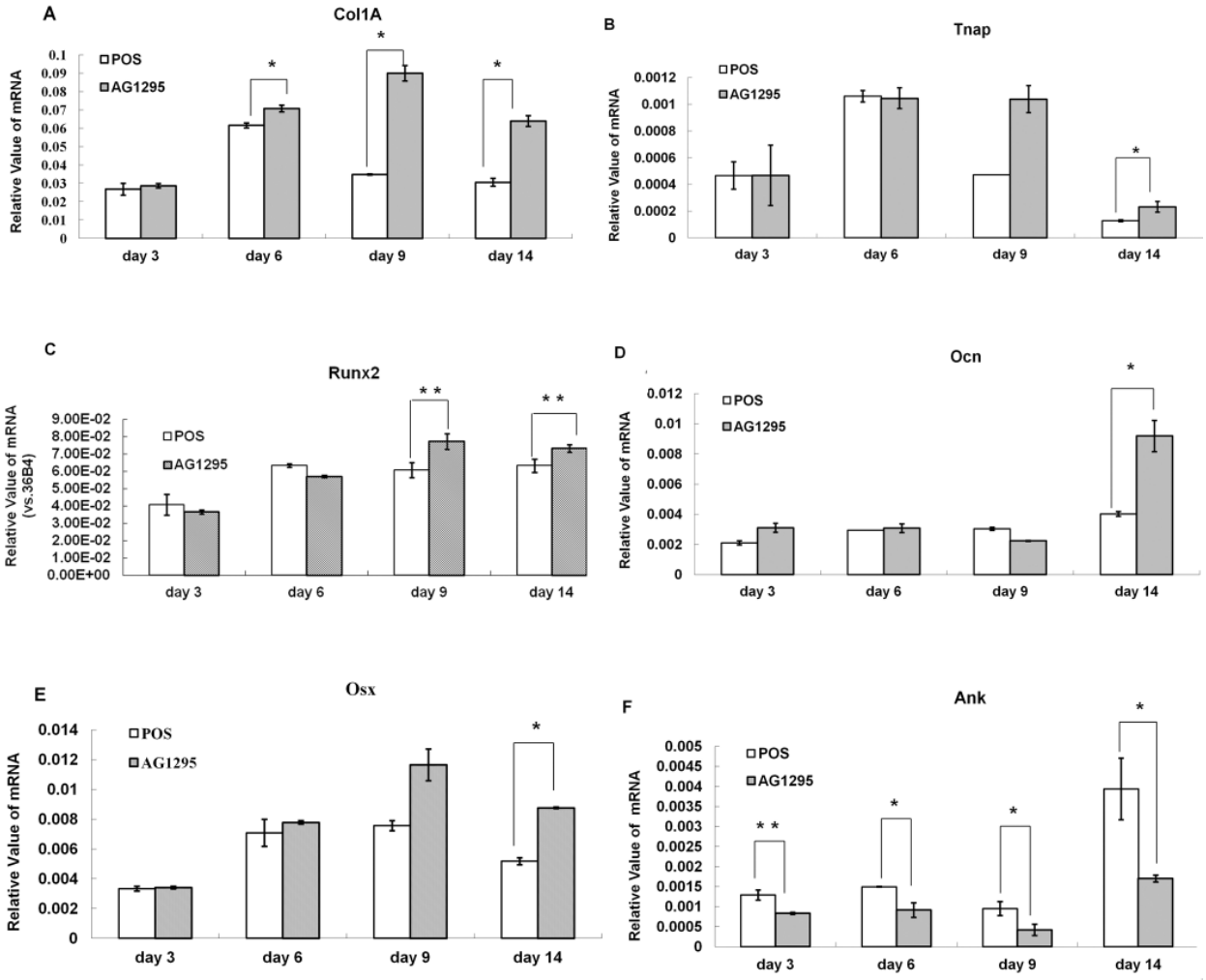

Figure 3. Effect of PDGF receptor inhibition on osteogenic marker gene expression assessed by quantitative real-time reverse transcription-polymerase chain reaction. Cells were grown in osteogenic media containing 0.1% DMSO (POS.) or 20 μmol/L Tyrphostin AG1295 for 3, 6, 9, and 14 days. Total RNA was extracted and measured by quantitative real-time polymerase chain reaction for *Col1A*, *Tnap*, *Runx2*, *Ocn*, *Osx*, and *Ank* mRNA expression. mRNA expression levels were normalized to 36B4. Results are expressed as mean arbitrary units ± S.D. ($n = 3$). Asterisks indicate statistically significant differences between groups ($^{*}\ p < 0.05$; $^{**}\ p < 0.01$).

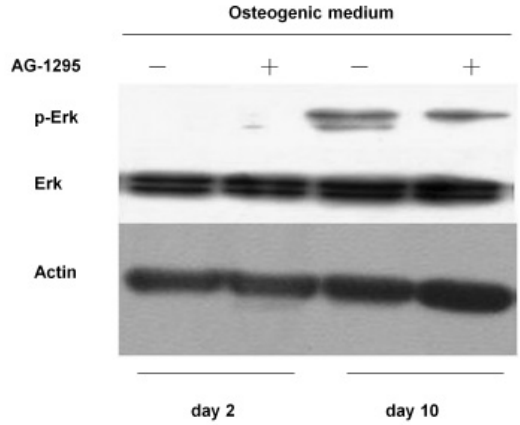

Figure 4. Inhibition of Erk1/2 by MC3T3-E1 cells treated with Tyrphostin AG1295. Cells were cultured in osteogenic media for 2 and 10 days in the presence of 0.1% DMSO or 20 μmol/L Tyrphostin AG1295, and cells were cultured in basic medium containing 0.1% DMSO alone as a negative control. At the appointed time, protein extraction and Western blotting were performed.

AG-1295 also increased the expression of most osteogenic markers. These findings suggest that PDGFR signaling suppresses osteoblast differentiation. The current findings differ from those of a previous study by Kumar *et al.*, in which another PDGFR inhibitor, AG-1296, failed to affect the osteogenic differentiation of human mesenchymal stem cells (MSCs). Differences may have arisen because of the different cell lines and PDGFR inhibitors used. Human MSCs give rise to osteoblastic lineages when cultured in specific differentiation media. Both MC3T3-E1 and human MSCs are commonly used in studies of osteogenic differentiation. They also have a similar mineralization process. Differences in results may be due primarily to different PDGFR inhibitors. AG-1296 has been found to inhibit PDGFR-α longer and greater than AG-1295, while AG-1295 is a specific inhibitor of PDGFR-β (*18,19*). Nemoto *et al.* (*20*) indicated that during formation of mineralized nodules PDGFR-β remained slightly elevated while PDGFR-α remained slightly depressed. Therefore, the current findings also suggest that PDGFR-β rather than PDGFR-α signaling is involved in the regulation of osteoblast differentiation.

The detailed mechanism for regulation of mineralization by PDGFR signaling is not clear at present. Several previous studies have shown that the Erk pathway, a downstream effector of PDGFR signaling, is essentially involved in regulation of matrix mineralization. Higuchi *et al.* (*21*) demonstrated that Erk inhibitor PD98059 promoted osteoblastic differentiation induced by BMP-2 in C2C12 pluripotent mesenchymal cells. Chaudhary and Avioli reported that Erk activation by PDGF factor BB or fibroblast growth factor-2 suppressed type I collagen expression in MC3T3-E1 cells (*22*). These findings suggest that the Erk pathway is a negative regulator of osteoblastic differentiation. To explore the possible role AG1295 plays in osteogenesis, its effect on the Erk1/2 pathway was investigated in MC3T3-E1 cells. As expected, the phosphorylation activation of Erk1/2 was significantly suppressed by 20 μmol/L Tyrphostin AG-1295 in MC3T3-E1 cells at day 10, which suggests that AG-1295 might promote osteoblast differentiation by suppressing the activation of Erk1/2, a downstream factor of the PDGFR pathway.

In summary, the present study has demonstrated that inhibition of PDGFR signaling by Tyrphostin AG-1295 significantly promoted osteogenesis *via* the Erk1/2 pathway. Findings suggest that PDGFR-β signaling might play an important role in osteoblast differentiation.

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Overexpression of hepatocyte growth factor receptor in scleroderma dermal fibroblasts is caused by autocrine transforming growth factor β signaling

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Summary **Cutaneous fibrosis seen in systemic sclerosis (SSc) is caused by fibroblast activation and abnormal collagen accumulation due to 'autocrine transforming growth factor (TGF)-β/Smad signaling'. Hepatocyte growth factor (HGF) may have therapeutic value against SSc, because of its inducible effect on the expression of matrix metalloproteinase (MMP)-1. Previous studies indicated SSc dermal fibroblasts overexpress HGF receptor c-met, which suggest specific and effective induction of MMP-1 in SSc fibroblasts caused by HGF treatment. However, the exact mechanism of c-met overexpression in SSc cells was hardly investigated. We hypothesized that such c-met overexpression is also caused by autocrine TGF-β/Smad signaling. Expression of c-met protein in cultured SSc dermal fibroblasts was significantly up-regulated compared with that in normal fibroblasts. Ectopic TGF-β stimulation induced c-met synthesis in normal fibroblasts, while a TGF-β knockdown normalized the up-regulated c-met levels in SSc fibroblasts. Furthermore, we found the c-met promoter contains a putative binding site for Smads, and the binding activity of Smad2/3 to the c-met promoter was constitutively up-regulated in SSc fibroblasts as well as in normal fibroblasts treated with exogenous TGF-β1. Taken together, c-met may be overexpressed due to autocrine TGF-β/Smad signaling in SSc. Considering that HGF has an antifibrotic effect, such c-met overexpression in SSc fibroblasts may be a negative feedback against cutaneous fibrosis. Clarifying the mechanisms of c-met overexpression and controlling the HGF/c-met pathway may lead to a new therapeutic approach for this disease.**

Keywords: Immunoblotting, fibrosis, extracellular matrix

1. Introduction

Systemic sclerosis (SSc) is one of the autoimmune disorders characterized by tissue fibrosis of the skin as well as internal organs. The activation of fibroblasts is thought to be responsible for the tissue fibrosis *via* the abnormal accumulation of extracellular matrix (ECM), mainly collagen (*1,2*). Although the mechanism of fibroblast activation is still unknown, many of the characteristics of SSc fibroblasts resemble those of transforming growth factor (TGF)-β1 stimulated normal fibroblasts (*3,4*). The principal effect of TGF-β1 on mesenchymal cells including fibroblasts is ECM production. Cultured SSc fibroblasts overexpress various ECM components, mainly type I collagen (*5,6*), as well as display constitutively up-regulated phosphorylation and promoter-binding activity of Smad, a mediator of TGF-β signaling (*7-9*). Also, our previous findings described below suggest that the activation of dermal fibroblasts in SSc is due to stimulation by 'autocrine TGF-β signaling'; (*i*) The blockade of TGF-β signaling with neutralizing antibody abolished the overexpression of collagen mRNA in cultured SSc fibroblasts (*10*), and (*ii*) the TGF-β-responsive element of α2(I) collagen promoter in normal fibroblasts and

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the sequence involved in the intrinsic up-regulation of α2(I) collagen promoter activity in SSc fibroblasts are both bp −376 ~ −108 sites (*11,12*). Thus, the intrinsic up-regulation of collagen genes seen in SSc fibroblasts utilizes a TGF-β1/Smad-dependent pathway.

Recently, hepatocyte growth factor (HGF) has attracted attention for its therapeutic value in treating various diseases. HGF regulates cell growth, motility and morphogenesis by binding to the receptor called c-met (*13*). HGF may also have an anti-fibrotic effect inducing the expression of matrix metalloproteinase (MMP)-1 in dermal fibroblasts (*14,15*). We previously compared the direct effect of HGF on the expression of type I collagen and MMP-1 in normal and SSc cultured dermal fibroblasts. HGF reduced type I collagen expression in SSc fibroblasts, but not in normal cells (*14*). On the other hand, MMP-1 expression was increased by HGF in both cells, but HGF had stronger effects in SSc fibroblasts than normal fibroblasts. We concluded that HGF reduces type I collagen accumulation only in SSc fibroblasts by the effective induction of MMP-1 in these cells, because of the overexpression of c-met. C-met overexpression in SSc fibroblasts was demonstrated by immunohistochemistry *in vivo* and immunoblotting *in vitro* (*14,16*). Thus, c-met overexpression in SSc fibroblasts is likely to be the key event to express the anti-fibrotic effect of HGF in this disease. However, its mechanism has not been investigated well. Clarifying the regulatory mechanisms of HGF/c-met signaling in SSc may contribute to further understanding of this disease and lead to a new therapeutic approach. We hypothesized that such c-met overexpression is also caused by autocrine TGF-β/Smad signaling. This study was undertaken to evaluate the hypothesis, and to clarify its mediators.

2. Materials and Methods

2.1. *Cell culture*

Human dermal fibroblasts were obtained by skin biopsy from the affected areas (dorsal forearm) of 4 patients with diffuse cutaneous SSc and < 2 years of skin thickening as described previously (*17*). Control fibroblasts were obtained by skin biopsy from 4 healthy donors (*18*). Institutional review board approval and written informed consent were obtained according to the Declaration of Helsinki. Primary explant cultures were established in 25-cm^2 culture flasks in modified Eagle's medium (MEM) supplemented with 10% fetal calf serum (FCS) and an Antibiotic-Antimycotic (Invitrogen, Carlsbad, CA, USA). Monolayer cultures independently isolated from different individuals were maintained at 37ºC in 5% CO_2 in air. Fibroblasts between the third and sixth subpassages were used for experiments. Before experiments, cells were serum-starved for 12-24 h.

2.2. *Cell Lysis and immunoblotting*

Fibroblasts were cultured until they were confluent, then cell lysates (normalized for protein concentration) were analyzed by immunoblotting as described previously (*19*). The antibodies for c-met and β-actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

2.3. *RNA isolation and quantitative real-time polymerase chain reaction (PCR)*

Total RNA was extracted from culture cells with ISOGEN (Nippon Gene, Tokyo, Japan). First-strand cDNA synthesis and quantitative real-time PCR with Takara Thermal Cycler Dice (TP800) were performed as described previously (*19*).

Primer sets for c-met and GAPDH were purchased from SABiosciences (Frederick, MD). DNA was amplified for 40 cycles of denaturation for 5 sec at 95° C and annealing for 30 sec at 60°C. The relative fold changes of c-met and GAPDH were calculated by a standard curve method. For each gene of interest, we used at least 3 independent samples.

2.4. *Transient transfection*

The cells were transfected with TGF-β1 siRNA or control siRNA (Santa Cruz Biotechnology) mixed with Lipofectamine RNAiMAX (Invitrogen, Carlsbad, CA, USA) as a transfection reagent when cells were plated, and incubated for 96 h at 37°C in 5% CO_2 (*18*). Control experiments showed > 80% knockdown efficiency (data not shown).

2.5. *DNA affinity precipitation assay*

Three oligonucleotides containing biotin on the 5' nucleotide of the sense strand were prepared as described previously (*20*). The sequences of these oligonucleotides are as follows: (*i*) c-met promoter oligo, 5'-ACAGCACG CGAGGCAGACAGACACGTGCTGGGGCGG, which corresponds to bp −364 to −329 positions of the human c-met promoter; (*ii*) positive control CAGA oligo, 5'-TC GAGAGCCAGACAAGGAGCCAGACAAGGAGCC AGACACTCGAG, positive control with a trimer of the CAGA motif; (*iii*) negative control TATA oligo, 5'-ACA GCACGCGAGGTATATATATACGTGCTGGGGCGG, which has a mutated CAGA motif of the c-met promoter oligo. These oligonucleotides were annealed to their respective complementary oligonucleotides, and double-stranded oligonucleotides were gel-purified and used. Cell lysates were obtained using lysis buffer (*21*). Poly (dI-dC) competitor was incubated with the cell lysates, followed by incubation with each double-stranded oligonucleotide. After incubation, streptavidin-agarose (Sigma, Saint Louis, MS) was added to the reaction

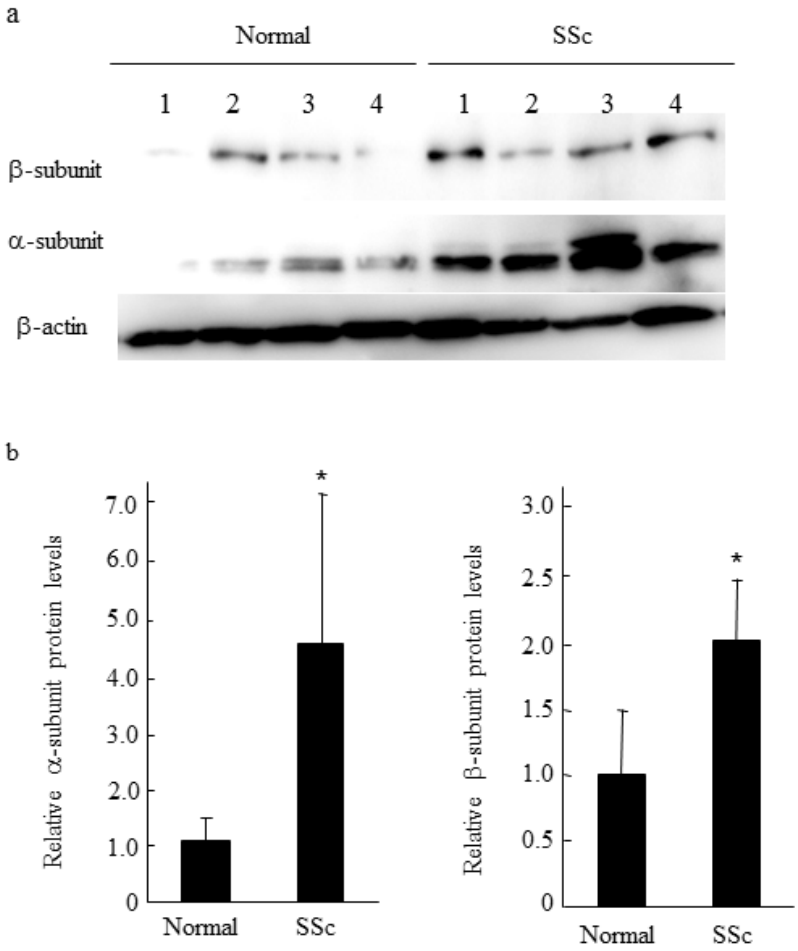

Figure 1. Levels of c-met protein synthesis in cultured dermal SSc fibroblasts. (a) The representative results of immunoblotting for lysates from 4 normal and 4 systemic sclerosis (SSc) fibroblasts. Cells were cultured until they were confluent, and then incubated for an additional 24 h under serum starvation conditions. Cell lysates were subjected to immunoblotting with antibodies against c-met or β-actin. The α- and β-subunits are indicated. **(b)** Expression of the α-subunit (left) or β-subunit (right) quantitated by scanning densitometry and corrected for levels of β-actin in the same samples are shown relative to those in normal fibroblasts (1.0). * $p < 0.05$ as compared with the values in samples from normal fibroblasts.

and incubated. The protein-DNA-streptavidin-agarose complex was washed and loaded onto a sodium dodecyl sulfate-polyacrylamide gel. Detection of Smad2/3 was performed by immunoblotting with monoclonal Smad2/3 antibody (BD Biosciences, Franklin Lakes, NJ, USA).

2.6. *Statistical analysis*

Data are expressed as the mean ± S.D. of at least three independent experiments. Statistical analysis was carried out with a Mann-Whitney *U*-test for comparison of medians. *p* values less than 0.05 were considered significant.

3. Results

3.1. *C-met protein synthesis in cultured SSc dermal fibroblasts*

As an initial experiment, we compared c-met protein expression levels between cultured normal and SSc fibroblasts by immunoblotting and confirmed the previous results (*14*). C-met is known to consist of an α- and β-subunit. The results showed that the amount of both subunits in the cell lysates from SSc fibroblasts was increased compared with that from normal cells (Figure 1a). When quantitated, the protein expression of α- or β-subunit was significantly increased about 2.0-fold or 4.3-fold in SSc fibroblasts compared to normal cells, respectively (Figure 1b). These data indicated that c-met protein synthesis was significantly and constitutively increased in cultured SSc dermal fibroblasts, as described previously (*14*).

3.2. *C-met protein expression induced by stimulation with TGF-β*

If autocrine TGF-β stimulation is the main cause of the constitutive up-regulation of c-met in SSc fibroblasts, exogenous TGF-β1 may increase c-met expression in normal fibroblasts. To test this possibility, we investigated the effect of exogenous TGF-β1 on the expression of

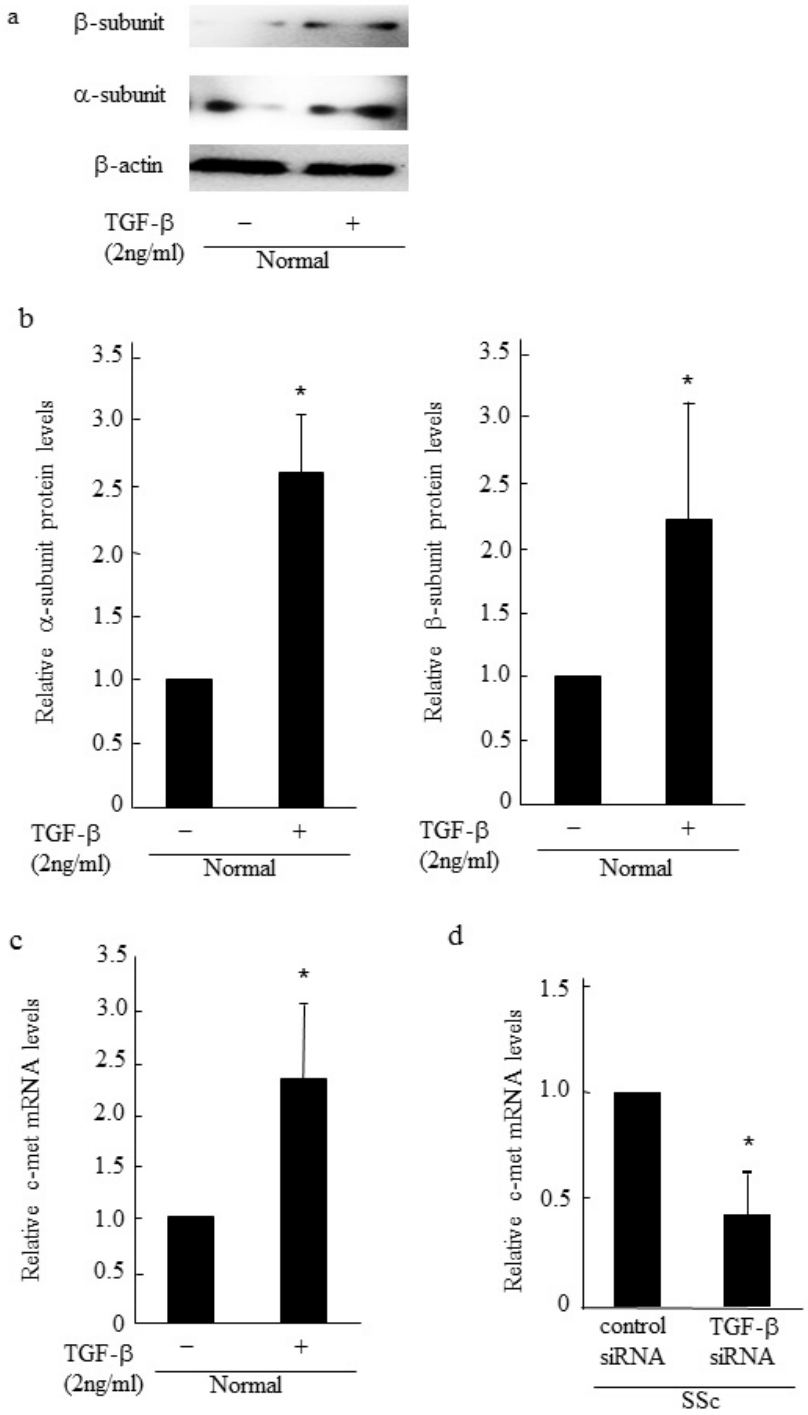

Figure 2. The effect of TGF-β1 on c-met expression in cultured dermal fibroblasts. (a) Cells were cultured until they were confluent, and then incubated for an additional 24 h under serum starvation conditions. Cells were incubated in the presence or absence of 2 ng/mL TGF-β1 for 24 h. Cell lysates were subjected to immunoblotting with antibodies against c-met or β-actin. **(b)** Expression of the α-subunit (left) or β-subunit (right) quantitated by scanning densitometry and corrected for levels of β-actin in the same samples are shown relative to those in untreated fibroblasts. Values in untreated fibroblasts were set at 1. * $p < 0.05$ as compared with the values in untreated fibroblasts. **(c)** Relative amounts of c-met transcripts (normalized with GAPDH) in fibroblasts stimulated with or without TGF-β1 (2 ng/mL) for 24 h were determined by real-time PCR. Values in untreated fibroblasts were set at 1. **(d)** SSc fibroblasts were transfected with control or TGF-β1 siRNA. After 96 h, c-met mRNA levels were determined by real-time PCR. * Values in fibroblasts transfected with control siRNA were set at 1. * $p < 0.05$ as compared with the value in cells transfected with control siRNA.

c-met protein and mRNA. Normal fibroblasts were cultured until they were confluent, and then incubated for an additional 24 h under serum starvation conditions. Cells were subsequently incubated for 24 h with or without 2 ng/mL TGF-β1 treatment. As shown in Figure 2a, the expression of c-met was increased by exogenous TGF-β1 stimulation in normal fibroblasts. In addition, the densitometric analysis revealed that overexpression of

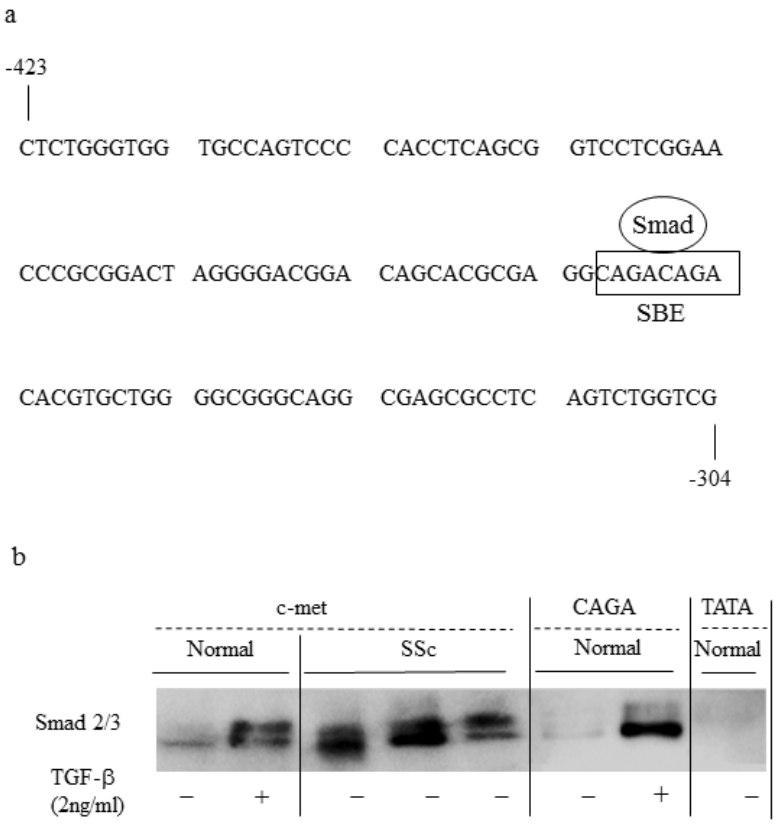

Figure 3. DNA-binding activity of Smad2/3 to c-met promoter in SSc fibroblasts. (a) Nucleotide sequence of c-met promoter region from bp −423 to −304 position. Putative Smad binding elements (SBE) which consisted of 2 CAGA motifs are boxed. **(b)** Comparison of the binding activity of Smad2/3 to the c-met promoter between normal and SSc fibroblasts by DNA affinity precipitation assay. Cells were incubated in presence or absence of 2 ng/mL TGF-β1 for 1 hour. Cell lysates were prepared and incubated with biotin-labeled oligonucleotides as described in Materials and Methods. Proteins bound to each nucleotide were isolated with streptavidin-agarose beads, and Smad2/3 was detected using immunoblotting. c-met; c-met promoter oligo, CAGA; positive control CAGA oligo containing CAGA motif, TATA; negative control TATA oligo with mutated putative SBE of c-met promoter.

the α- and β-subunit protein was statistically significant (Figure 2b). Also, quantitative real-time PCR showed c-met mRNA was elevated significantly by treatment with 2 ng/mL TGF-β1 (Figure 2c). Thus, stimulation of exogenous TGF-β increases the expression of c-met protein and mRNA in normal fibroblasts.

Moreover, to prove the involvement of TGF-β1 stimulation in c-met overexpression seen in SSc fibroblasts, we determined the effect of TGF-β1 siRNA. SSc fibroblasts treated with the siRNA showed a significant reduction in c-met mRNA expression (Figure 2d), which is consistent with normalization of abnormally increased collagen expression by TGF-β1 neutralizing antibody in SSc fibroblasts as described in the introduction (*10*). These results indicate that the overexpression of c-met as well as type I collagen in SSc fibroblasts is a result of stimulation by autocrine TGF-β activation.

3.3. *The promoter binding activity of Smad to c-met promoter in normal dermal fibroblasts treated with TGF-β1 and in SSc fibroblasts*

To further elucidate the detailed mechanisms involved in activation of c-met transcription by autocrine TGF-β signaling in SSc fibroblasts, we compared the DNA binding ability of endogenous Smad to the c-met promoter in normal and SSc fibroblasts using a DNA affinity precipitation assay.

We found that the c-met promoter has 2 tandem of CAGA sequences, known as the Smad binding element (SBE) (*22*), at the bp −351 ~ −344 position (Figure 3a). Fibroblasts were serum-starved for 24 h and treated with 2ng/mL TGF-β1 for 1 h. C-met promoter oligos were designed to correspond to bp −364 to −329 positions of the human c-met promoter, containing the putative SBE. As a positive control, we used the positive control CAGA oligo, which also contains the trimer of SBE (*8*). We also used a negative control (TATA oligo), in which the CAGA motif of c-met promoter oligo was mutated to TATA. As shown in Figure 3b, the results showed that Smad2/3 bound to the c-met promoter oligo strongly after TGF-β1 treatment in normal cells (lanes 1 and 2). SSc fibroblasts showed constitutive binding of Smad2/3 even without exogenous TGF-β stimulation (lanes 3-5). The positive control CAGA oligo could bind Smad2/3 as reported previously (*23*), whereas the negative control TATA oligo did not

show Smad2/3 binding. These results suggest that Smad2/3 bound to this site of the c-met promoter in an inducible and specific manner and supports the notion that TGF-β1/Smad mediates c-met overexpression in SSc fibroblasts.

4. Discussion

In this study, we have presented two major findings. First, as reported previously (*14*), SSc fibroblasts constitutively overexpress c-met. Exogenous TGF-β induces c-met expression in cultured human dermal fibroblasts at the mRNA level. Additionally, TGF-β knockdown by TGF-β1 siRNA decreased c-met mRNA in cultured SSc dermal fibroblasts which have autocrine TGF-β signaling. These results suggest that TGF-β plays a major role in c-met overexpression in SSc dermal fibroblasts.

Secondly, our report first indicated such overexpression of c-met in SSc fibroblasts is mediated by the Smad pathway. The DNA affinity precipitation assay revealed that c-met promoter contains a putative SBE, and the binding activity of Smad2/3 to the c-met promoter was constitutively increased in SSc fibroblasts, to a similar degree to that in normal fibroblasts treated with exogenous TGF-β1. As described above, the binding activity of endogenous Smad to α2(I) collagen promoter was also up-regulated remarkably in SSc fibroblasts compared with normal fibroblasts (*24*). Signals of TGF-β1 from the receptor to the nucleus are mediated by Smad proteins. The activated TGF-β receptor type I directly phosphorylates Smad2/3. Once activated, Smad2/3 associates with Smad4 and translocates to the nucleus, where the complex binds to the SBE in the promoter of target genes, resulting in modulation of their transcriptional activities. Thus, increased Smad binding to the c-met promoter in SSc fibroblasts without exogenous stimulation indicates that c-met overexpression in SSc fibroblasts results from stimulation of autocrine TGF-β signaling. Considering that HGF has an anti-fibrotic effect, such c-met overexpression in SSc fibroblasts may be a negative feedback against fibrosis of the skin.

Although a therapeutic effect of cyclophosphamide, prednisolone, or methotrexate therapy on the fibrosis of SSc (*25,26*) has been reported, various and considerable adverse effects of these treatments have to be a concern (*27*). On the other hand, as described above, the anti-fibrotic effect of HGF may be limited to fibrotic lesions of SSc because of c-met overexpression (*14*), indicating a less adverse effect. Clarifying the mechanisms of c-met overexpression and controlling the HGF/c-met pathway may lead to a novel therapeutic approach for this disease. The effect of other cytokines including TGF-β2 or -β3 on c-met expression should be examined in the future.

Acknowledgements

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Case Report

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Type I aortic dissection in a patient with human immunodeficiency virus infection

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Summary **The use of highly active antiretroviral therapy in patients with HIV infection has significantly reduced HIV-related infectious complications and improved their survival. With effective antiretroviral therapy, cardiovascular disease has gained prominence as a cause of morbidity and mortality in HIV-infected persons. Aortic dissection is an uncommon but potentially fatal disease with catastrophic complications. The spread of AIDS is a major public health problem in China, but there is scant literature regarding the clinical outcome for HIV/AIDS patients with aortic dissection in China. This case report describes a patient with HIV and type I aortic dissection who survived without surgical repair. This report is provided to describe a detailed and successful outcome for a patient with type I aortic dissection and HIV in China.**

Keywords: Aortic dissection, acquired immunodeficiency syndrome

1. Introduction

Human immunodeficiency virus (HIV) infection is characterized by a chronic disease process with systemic multiorgan involvement (*1*). In the early years of the acquired immunodeficiency syndrome (AIDS) epidemic, many patients suffered and died from serious opportunistic infection partly because of their compromised immune system. The use of highly active antiretroviral therapy (HAART) in patients with HIV infection has significantly reduced HIV-related infectious complications and improved their survival. This improvement, combined with the metabolic effects of antiretroviral treatment, has increased the risk of cardiovascular disease (*2*). HIV patients share many cardiovascular risk factors with the general population, but they also share factors specific to their condition that include the HIV virus itself, HIV replication, chronic inflammation, and exposure to HAART (*3,4*). Cardiovascular complications occur in a significant number of such patients and are the immediate cause of death in some. The spectrum of cardiovascular complications of AIDS that may be depicted at imaging includes dilated cardiomyopathy, pericardial effusion, pulmonary hypertension, endocarditis, thrombosis, embolism, vasculitis, coronary artery disease, aneurysm, atherosclerotic cardiovascular disease, and cardiac involvement in AIDS-related tumors (*5*). With effective antiretroviral therapy, cardiovascular disease has gained prominence as a cause of morbidity and mortality in HIV-infected persons. Aortic dissection is an uncommon but potentially fatal disease with catastrophic complications. It most commonly presents in the elderly population with a history of chronic hypertension. Rapid intervention is necessary as delay leads to higher mortality. Aortic dissection is a very uncommon cardiovascular complication reported in HIV-infected patients. Baciewicz *et al.* (*6*) reported a case of HIV and type I aortic dissection in which the patient was successfully treated with surgical repair. The spread of AIDS is a major public health problem in China; but there is scant literature regarding the clinical outcome for HIV/AIDS patients with aortic dissection in China. This case report describes a patient with HIV and type I aortic dissection who survived without surgical repair. This report is provided to describe a detailed and successful outcome for a patient with type I aortic dissection and HIV in China.

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2. Case report

A 46-year-old man infected with HIV who had a CD4 count of 150 cells per cubic millimeter was evaluated in November 2009 with complaints of searing pain in the chest, back, and abdomen. The patient denied being short of breath. He was diagnosed with HIV in 1995 and had received HAART (stavudine, lamivudine plus efavirenz) since 1997. The patient developed renal insufficiency and hypertension in 2008 and had been on hemodialysis since then. A physical examination revealed a blood pressure of 211/115 mmHg in both arms. The patient did not smoke or drink and he did not have dyslipidemia or hyperglycemia. He was negative for both HBsAg and HCVAb. On admission, a chest computerized tomography (CT) scan revealed a type I aortic dissection beginning in the ascending aorta and extending to the left iliac artery (Figure 1). Surgical repair was not done because the dissection involved the entire aorta. Medical treatment included aggressive control of the patient's blood pressure and heart rate. He continued to receive antiretroviral therapy (stavudine, lamivudine plus efavirenz) and undergo hemodialysis. A subsequent CT scan performed one month later revealed no change in residual dissection of the aorta. The thoracic and abdominal aorta diameters have remained stable (Figure 2). The patient continued to be followed up regularly, but he died on July 7, 2010 (8 months after the aortic dissection appeared) because of cardiac complications.

3. Discussion

With the further spread of AIDS worldwide and a dramatic increase in the life expectancy of HIV-infected patients treated with effective antiviral regimens, an increasing number of patients live with the illness but more than 10% experience cardiovascular manifestations (*7*). Before the advent of HAART, cardiac manifestations in HIV patients mainly included cardiomyopathy, pancarditis, and pulmonary hypertension leading to heart failure, conduction system abnormalities, and neoplastic infiltration (*8*). In the post-HAART era, acute coronary events by far outnumber all other cardiovascular complications of HIV (*7*). Cardiovascular prevention is required in more than one-half of HIV-infected/treated patients for HAART to be reliably effective (*7*). As the prognosis for HIV patients continues to improve, this rate is likely to increase. This increase has been attributed to aging along with a resulting increase in risk factors such as hypertension and diabetes, as well as HAART regimens that include stavudine or protease inhibitors (PIs). All medications in this latter class have a reported association with hyperlipidemia, hyperglycemia, and truncal obesity (*9*). Atherosclerotic cardiovascular disease has become more frequent with the use of HAART. Studies indicate that new-generation PIs such as darunavir/ritonavir (*10*) and atazanavir/ritonavir (*11*) are relatively less likely to lead to dyslipidemia. The integrase inhibitor raltegravir and CCR5 receptor antagonist inhibitor maraviroc have a better lipid and glycemic profile than older PIs and thymidine analogues (*12*). In addition, HIV has been found to directly affect vascular biology, resulting in an increased risk of cardiovascular disease compared to uninfected persons (*13*). The current patient had received stavudine-based antiretroviral

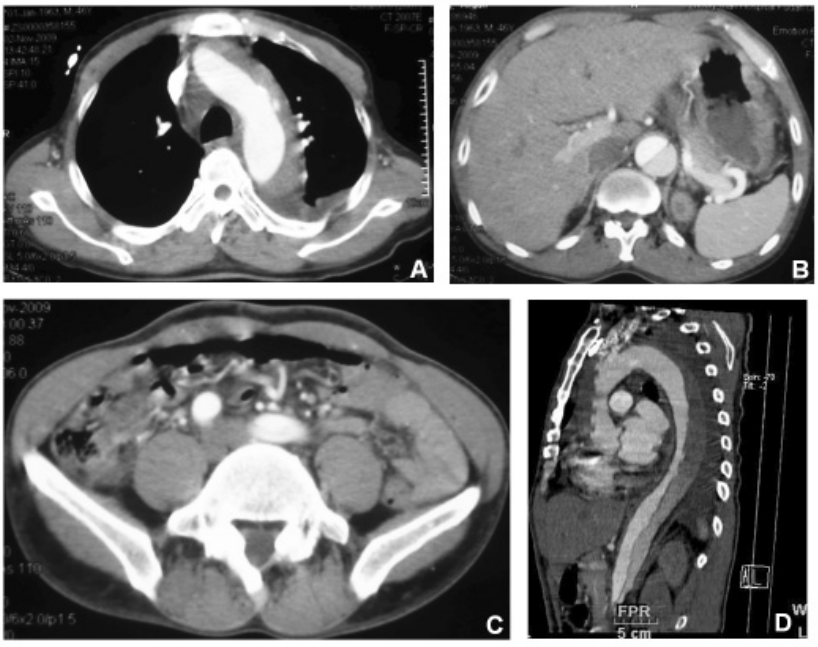

Figure 1. CT scan performed on November 2nd 2009 showing a type I aortic dissection beginning in the ascending aorta and extending to the left iliac artery (A, B, C, and D).

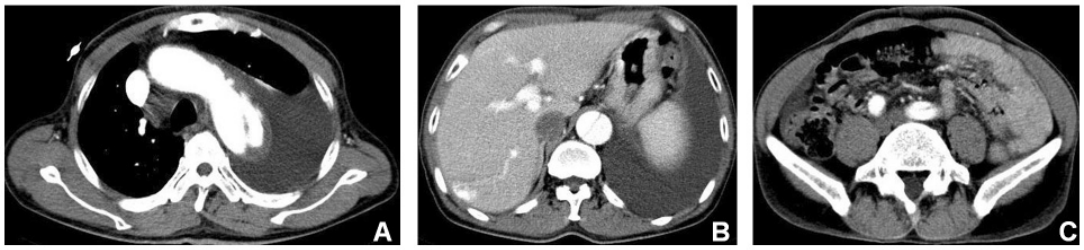

Figure 2. CT scan performed on December 4th 2009 showing no change in the dissection of the aorta. The thoracic and abdominal aorta diameters have remained stable (A, B, and C).

therapy for nearly 13 years, he had developed renal insufficiency and hypertension, and he had been on hemodialysis since 2008. The exact cause of renal failure in this patient is unknown, though it may be associated with HIV infection. Kidney function is abnormal in up to 30% of HIV patients; AIDS-related kidney disease has become a relatively common cause of end-stage renal disease requiring dialysis (*14*). The origins of aortic dissection in the current patient appear to be multifactorial and related to high blood pressure, hemodialysis treatment, HIV infection, as well as to the adverse reactions to antiretroviral drugs. The prevalence of hypertension in HIV disease was estimated to be 20% to 25% before HAART but is now up to 74% in patients with HAART-related metabolic syndrome (*15*). Recent reports indicate that elevated blood pressure may be related to PI-induced lipodystrophy and metabolic disorders and especially to elevated fasting triglycerides (*8*). Acute or chronic renal failure also contributes to hypertension (*8*). In the current patient, hypertension may have been associated with renal failure and adverse reactions to antiretroviral therapy and may have been the most significant cause of aortic dissection.

Acute aortic dissection is a medical emergency with high morbidity and mortality requiring prompt diagnosis and treatment. A high degree of caution is required for its successful diagnosis as presenting symptoms are so variable that dissection may be overlooked in up to 39% of cases (*16*). Rapid advances in noninvasive imaging technology have facilitated the early diagnosis of this condition and should be considered in the differential diagnosis of any patient with chest, back, or abdominal pain. Dissections may involve the ascending aorta alone, the descending thoracic and abdominal aorta alone, or the entire aorta. An aortic dissection is serious because it may rupture, causing life-threatening internal bleeding. The risk of death depends on the extent of the dissection. The risk is highest for those dissections involving the ascending aorta. Emergency surgery is the treatment for patients with a type A dissection while optimal medical therapy is appropriate for patients with an uncomplicated type B dissection. The medical treatment of an aortic dissection includes aggressive control of blood pressure and heart rate while the aorta heals. An adequate beta-blockade is the cornerstone of medical therapy. The literature mentions one HIV-infected patient who underwent surgical repair of a type I aortic dissection (*6*). Select patients with a type I aortic dissection and HIV infection are candidates for surgical repair (*6*). One study indicates that perioperative morbidity and mortality rates are high in HIV patients undergoing abdominal aortic surgery (*17*). HIV infection itself does not seem to increase perioperative morbidity and mortality in cardiac surgery and major cardiac surgery does not negatively affect the course of HIV infection (*18*). Once the acute dissection has healed, adequate control of blood pressure may eliminate the need for surgery. Patients who survive acute aortic dissection need long-term medical therapy with beta-blockers and appropriate serial imaging follow-up. Lifelong monitoring of the diameter of the aorta is required because a previously dissected aorta may enlarge and rupture.

Because of the nature of the viral infection and the possible mode of viral transmission, many surgeons remain reluctant to perform invasive procedures on patients with HIV infection in China. Presently, there are no definitive or specific treatment guidelines from different surgical societies regarding surgical management of patients with HIV infection. The current patient with type I aortic dissection did not undergo emergency surgical repair but he did survive acute aortic dissection and he received long-term medical therapy with beta-blockers and appropriate serial imaging follow-up.

As the epidemic progresses and new treatments help increase the long-term survival of AIDS patients, cardiovascular complications will become more common. Although HIV infection can now be treated effectively with a combination of antiretroviral medications, cardiovascular diseases present new challenges for the management of persons infected with HIV in China. Patients with aortic dissection have a high risk of an adverse outcome and need to be managed aggressively in hospital and over the long-term with frequent follow-ups (*19*). Future advances in this vein include the early diagnosis and optimal treatment of aortic dissection in HIV-infected patients.

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Commentary

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"Knowledge into action" – Exploration of an appropriate approach for constructing evidence-based clinical practice guidelines for hepatocellular carcinoma

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Summary **With the development of evidence-based medicine (EBM), the concept of "transfer of current best evidence into clinical decision-making" has garnered substantial attention worldwide. As such a good tool, many clinical practice guidelines (CPGs) for hepatocellular carcinoma (HCC) have been published worldwide under the guide of current best evidence. Our study did a systematic evaluation of the current 17 guidelines for HCC worldwide, which found that the appropriate constructing approach is the most important factor that influences guidelines implementation. Three factors of organizations or bodies drafting the guideline, exploration for achieving current best evidence, and purpose of constructing evidence-based CPGs for HCC should be paid close attention to. In order to achieve the current best evidence and promote evidence-based CPGs to be widely accepted and fully implemented, we recommend to conduct a systematic approach with 4 steps of global guidelines assessment, systematic literature review, experts' consensus and draft implementation, as well as implementation evaluation and periodic update in constructing and implementing evidence-based CPGs for HCC.**

Keywords: Hepatocellular carcinoma (HCC), clinical practice guidelines (CPGs), standardized management of care, evaluation

1. Introduction

Evidence-based medicine (EBM), which was defined as "the integration of current best evidence with clinical expertise and patient values", could go back to mid-19th century Paris and earlier, remains a hot topic for clinicians, public health practitioners, purchasers, planners, and the public worldwide (*1*). The core of EMB is the conscientious, explicit, and judicious use of current best evidence in making decisions about the care of individual patients (*2*). About how to get "current best evidence", there have been many explorations in clinical practice during the past decades, such as, Team Oncology Medicine (*3*), and international registration of clinical trials (*4*). In recent years, the concept of "standardized management of care" has garnered substantial attention and has been fully implemented in several countries worldwide. Construction of a disease management guideline that specifies appropriate diagnoses and treatments based on scientific research evidence and collaborations between medical professionals involved in the treatment of a given condition is the key to standardized management of care (*5,6*). Clinical practice guidelines (CPGs) as a management model, have been used for many cancers worldwide. CPGs are a good tool for transferring research evidence into clinical practice as well as getting new evidence in the course of influencing practitioners' attitude and clinical decision-making. The evidence-based CPGs are expected to achieve the

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following goals with full implementation: a) assisting practitioners in appropriate clinical decision-making; b) improving quality of healthcare and outcomes for patients; and c) supporting and influencing regional or national policies for efficient resources allocation and better delivery systems (*7*).

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the third leading cause of cancer-related deaths in men. The incidence of HCC is highest in Middle Africa (15.8%), followed by Eastern Asia (14.1%) and Western Africa (10.6%) (*8*). In the past decade, several remarkable advances have been made in the management of HCC. More importantly, many CPGs for HCC have been published worldwide with the purpose of reducing incidence and mortality as well as improving healthcare quality for patients. Our study group did an English language literature search on the topic of guidelines or consensus for HCC published in the PubMed database during the period of 2001 to 2011. After a second screening, 46 articles were adopted from 3,008 hits to form 17 current guidelines for HCC around the world according to the selection criteria of credibility, influence, and being multi-faceted (*9*), including 5 guidelines from America, 7 from Asia, and 5 from Europe (Table 1). We did a systematic evaluation on 17 current guidelines for HCC, which found that these guidelines have both similarities and differences in terms of what organizations or bodies drafted the guidelines and the approach, applicability, content, and recent updates of the guidelines as well as in terms of diagnostic and treatment algorithms. The comparative analysis of projected goals and implementation of guidelines for HCC showed that evidence-based CPGs for HCC is urgently needed and the appropriate constructing approach is the most important factor that influences guideline implementation.

2. "3 W" – Factors influence implementation of CPGs for HCC

2.1. *"Who": Organizations or bodies drafting the guideline*

Of the current 17 guidelines, only the J-HCC Guideline (*10*) in Japan was constructed with the support of government, the other 16 guidelines were constructed by national academic societies. Of the guidelines drafted by an expert panel that consisted mainly of hepatologists, only the J-HCC Guideline and the APASL Guideline (*11*) specified an expert panel consisting of radiologists, statisticians, and other experts besides hepatologists. The absence of governmental support and lack of public health experts may lead to gaps in information on the management of HCC, especially with regard to appropriate prevention and surveillance measures, domestic health systems, health resources, income levels, and so on. The absence of the above important information may hamper patients from getting adequate information on prevention and early detection, as well as hamper health policy-makers from making optimal health resource distributions, such as establishing nationwide programs for prevention, screening and surveillance. Our study recommends that constructing evidence-based CPGs for HCC require the early participation of all related bodies, including health policy-makers. For a guideline to achieve wide acceptance and full implementation, health policy-makers need to be given clear messages about its potential impact in order to help them in efficient resource allocation and better delivery systems. The expert panel drafting CPGs should consist of not only clinicians, but also experts from health statistics, epidemiology, health policy, health economics, and so on.

Table 1. Current characteristic guidelines for HCC worldwide

Areas	No.	Years	Drafted by	Guidelines
America	1	2005	National Comprehensive Cancer Network	NCCN Guideline
	2	2005	American Association for the Study of Liver Disease	AASLD Guideline
	3	2007	American College of Surgeons	ACS Guideline
	4	2009	World Gastroenterology Organisation	WGO Guideline
	5	2010	United States National Cancer Institute	NCI (USA) Guideline
Asia	1	2003	Korean Liver Cancer Study Group and National Cancer Center	Korean Guideline
	2	2005	Japanese Ministry of Health, Labor, and Welfare	J-HCC Guideline
	3	2006	Saudi Gastroenterology Association	SGA Guideline
	4	2007	Japan Society of Hepatology	JSH Guideline
	5	2008	Asian-Pacific Association for the Study of the Liver	APASL Guideline
	6	2009	Asian Oncology Summit 2009	AOS Guideline
	7	2009	Chinese Society of Liver Cancer Chinese Society of Clinical Oncology Chinese Society of Hepatology Liver Cancer Study Group	Chinese Guideline
Europe	1	2001	European Association for the Study of the Liver	EASL Guideline
	2	2003	British Society of Gastroenterology	BSG Guideline
	3	2004	Belgian Association for the Study of the Liver	BASL Guideline
	4	2008	European Society for Medical Oncology	ESMO Guideline
	5	2008	Italian Southern Oncological Group	GOIM Guideline

2.2. *"Way": Exploration for achieving current best evidence*

Our study showed that CPGs should be the integration of the following three aspects in order to achieve current best evidence:

Evidence-based information. Of the current 17 guidelines, 5 guidelines were constructed by systematic literature analysis, which provided data-support recommendations on the management of HCC, and the other 12 guidelines were constructed by experts' consensus, which provided experts' experience-support recommendations on the management of HCC. According to levels of evidence (from level 1 to level 5, from high to low) from the Oxford Center for Evidence-Based Medicine (CEBM) (*12*), guidelines drafted based on a literature analysis have a different level of evidence (level 1 to level 4) than do guidelines drafted based on experts' consensus (level 5), but both are still in accordance with EBM. The management of HCC in Japan showed that consensus recommendations based on experts' experience could provide additional information for CPGs, which is especially helpful for up-to-date information (*9*). Our assessment of guidelines established by literature analysis or experts' consensus showed that both of them have advantages and disadvantages. Thus, we recommend that the best move is to construct evidence-based CPGs for HCC by combining a systematic literature review with experts' clinical experience.

Resource-based information. Of the current 17 guidelines, only the AOS Guideline (*13*) and the WGO Guideline (*14*) suggested providing different recommendations for countries with minimal resources, moderate resources, or extensive resources. It has been shown that although evidence-based CPGs constructed in resource-affluent countries define optimal goals and care, many measures could not be directly implemented in resource-constrained countries due to the lack of required fundamental infrastructure and resources (*15*). For example, many studies have proved that the combined testing of DCP and AFP or AFP-L3 could increase the sensitivity of HCC diagnosis, but DCP is currently approved just in Japan, Korea, and Indonesia (*9*). Thus, our study recommends that local resources in terms of health systems, medical technology, income levels, and other resources must be given full consideration in constructing evidence-based CPGs for HCC.

Population-based information. There are many geographic variations in the prevalence of HBV-related and HCV-related HCC as well as other cancerogenic factors. For example, HCV infection is the primary etiological factor in Western countries but HBV infection is the primary etiological factor in Asian countries except Japan. More importantly, the wide acceptance and full implementation of evidence-based CPGs for HCC will also be critically influenced by the understanding of the intended target population in the aspect of getting adequate information on prevention and early detection as well as keeping adequate cooperation with clinicians in clinical decision-making. Thus, from the perspective of cost-effectiveness analysis, our study recommends that population-based epidemiological information should be seriously taken into account in constructing evidence-based CPGs for HCC. It will be helpful in implementing CPGs, especially in the aspects of prevention, screening, surveillance, and appropriate selection of treatment modalities.

2.3. *"What": Purpose of constructing evidence-based CPGs for HCC*

The purpose of constructing evidence-based CPGs is to transfer current best evidence into clinical practice to improve medical treatment for patients with HCC. The establishment of evidence-based CPGs is just the beginning, not the end. How to promote CPGs to be widely accepted and fully implemented is one of the most important challenges. In the current 17 guidelines, only the J-HCC Guideline and the AASLD Guideline published their studies on awareness and influence of HCC guidelines, both of which showed that the HCC Guideline could benefit clinicians in clinical decision-making (*16,17*), but no other supporting data were found on the aspects of improving outcomes for patients and health policy-makers. The management of HCC in Japan has achieved remarkable results, which are attributed to a combination of quantitative and qualitative evaluation incorporated in the J-HCC Guideline (*18*). The J-HCC Guideline was first published in 2005 using systematic literature analysis of 7,192 publications on HCC and then revised in 2009 with the incorporation of new evidence. Prior to publication, a draft of the J-HCC Guideline was submitted for internal evaluation (the 2005 version was evaluated by 101 councilors of the Liver Cancer Study Group of Japan and the 2009 revision was evaluated by the 45th Japan Society of HCC) and external evaluation (the 2005 version was evaluated by an external review board and the 2009 revision was available on the Web to seek public comments). In addition, a questionnaire survey was conducted in 2006 to investigate the level of awareness and influence of the J-HCC Guideline among 2,279 members of the Liver Cancer Study Group of Japan and 689 primary care physicians in Osaka and Hyogo prefectures (*16*), which showed that more than 70% of clinicians have acknowledged the guideline, and part of the clinicians have changed their practices to follow it. Thus, our study recommends to actively explore an appropriate approach to promote guidelines for use on the basis of absorbing advanced experience from current well implemented CPGs worldwide, especially the systematic evaluation from the whole course of constructing and implementing CPGs for HCC.

3. "4 S" – Constructing evidence-based CPGs for HCC

Our study concludes a systematic approach for constructing and implementing evidence-based CPGs according to our comparative assessment on the current 17 guidelines for HCC worldwide. It could be divided into 4 steps (Figure 1):

Step 1: Global guidelines assessment to get advanced experience. During the past decade, many guidelines for HCC have been published worldwide and some recommendations have been widely accepted and fully implemented, such as, liver resection is most beneficial for solitary tumors in patients without cirrhosis, with post-resection 5-year survival rates of 41-74%, and liver transplantation for patients meeting Milan criteria (a solitary tumor ≤ 5 cm or up to 3 tumors ≤ 3 cm each) could obtain 5-year survival rates of 70-80% (*19*,*20*). So global guidelines assessment is necessary and could help us get advanced experience worldwide, especially for current criteria of diagnosis and treatment for HCC.

Step 2: Systematic literature review to get the native information for evidence-based, resource-based, and population-based situations. According to EBM, systematic literature review and analysis is one of most important ways to get the current best evidence, but it also should be noted that evidence-based CPGs established in resource-affluent countries defining optimal care and services have limited use in resource-constrained countries. In addition, there are many differences in the measures of prevention, screening, surveillance and appropriate selection of treatment modalities due to the variations of population-based epidemiology information and understanding of intended target populations. So systematic literature review as well as research on native health resources and population is necessary, it will be helpful in getting native information for evidence-based, resource-based and population-based situations, especially local resources in terms of health systems, medical technology, income levels, and so on, then benefit CPGs to be really widely accepted and fully implemented.

Step 3: Experts' consensus and draft implementation to get the evaluation information. Evidence-based CPGs constructed by systematic literature analysis could provide data-support recommendations, but some information, especially some of the most up-to-date information, is still lacking due to the factors of papers' publishing cycle, *etc*. The management of HCC in Japan has shown that experts' consensus-based recommendations could provide additional information for evidence-based CPGs, especially for some up-to-date information, so it is necessary to conduct internal evaluation among experts' in related areas before CPGs can be officially published. In addition, CPGs are a good tool for transferring research evidence into clinical practice, on the one hand, they are the scientific conclusion of current research evidence, and on the other hand, they could provide new evidence in the course

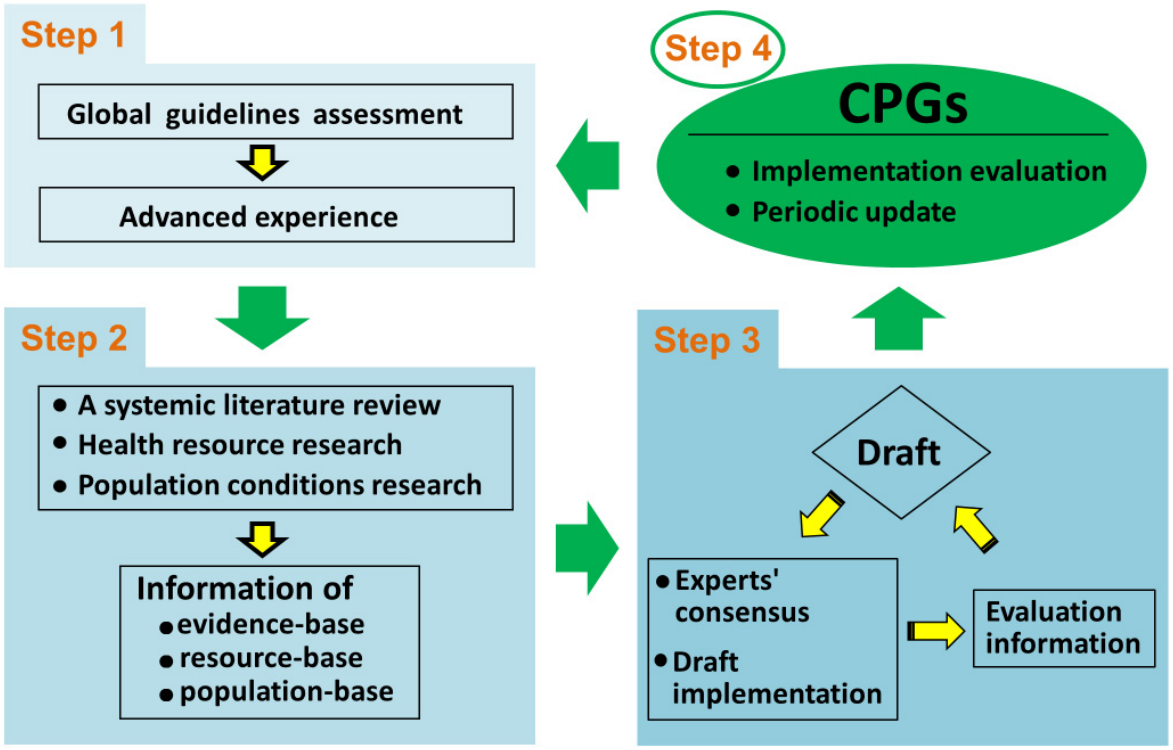

Figure 1. Approach for constructing and implementing evidence-based CPGs for HCC.

of implementation. So it is necessary to conduct draft implementation to get the most up-to-date evidence, especially the evaluation information about how CPGs influence clinical decision-making for clinicians and health resource distribution for health policy-makers, as well as outcomes for patients with HCC.

Step 4: Evaluation on guideline implementation and periodic update with incorporation of new evidence. After establishment of evidence-based CPGs for HCC, promotion of CPGs to be widely accepted and fully implemented is one of the most important challenges. Systematic evaluation is necessary to be conducted to examine the implementation effect of CPGs for HCC, including evaluation of awareness and influence of CPGs for clinicians, outcomes of adhering to CPGs for patients, efficient resource allocation for health policy-makers, and so on. In addition, evidence-based CPGs for HCC is in accordance with EBM, especially in achieving current best evidence to guide clinical practice, so evidence-based CPGs for HCC should be periodically updated with incorporation of new evidence every 3-4 years.

4. Conclusion

"Knowledge into action", with the development of EBM, evidence-based CPGs for HCC are urgently needed to transfer current best evidence into clinical practice to improve medical treatment for patients with HCC. In order to achieve current best evidence and promote evidence-based CPGs to be widely accepted and fully implemented, it requires the early participation of all stakeholders, including clinician, experts from health statistics, epidemiology, health policy, health economics, as well as health policy-makers; CPGs should be the integration of native information from evidence-based, resource-based and population-based situations; and systematic evaluation should also be conducted during the whole course of constructing and implementing CPGs for HCC. Based on the comparative assessment of current guidelines for HCC worldwide, we recommend conducting a systematic approach with 4 steps of global guidelines assessment, systematic literature review, experts' consensus and draft implementation, as well as implementation evaluation and periodic update in constructing and implementing evidence-based CPGs for HCC.

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