

Rubella virus sero-prevalence and associated factors among non-vaccinated pregnant women in Northwest Ethiopia

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Abstract

Background: Rubella virus infection during pregnancy is associated with adverse fetal outcomes and reproductive failures. In Ethiopia, little is known about the extent of the disease and rubella vaccination is not widely available. The main aim of this study was to assess the sero-prevalence of the rubella virus infection and its associated risk factors among pregnant women.

Methods: An institution based cross-sectional study was conducted in the antenatal clinics of Debre Markos and Debre Tabor hospitals of Amhara Region, Northwest Ethiopia from March to June 2015. Study participants were recruited until the calculated sample size was achieved at both hospitals. Data on socio-demographic and factors associated with rubella virus infection were collected through a structured questionnaire. A 5ml blood sample was also collected from all study participants and tested for Immunoglobulin (Ig) G and IgM antibodies against rubella virus infection using enzyme immune assay (EIA) test at the Amhara Regional Health Research Laboratory Center, Bahir Dar. Data were analyzed using SPSS version 21 and frequencies, chi-square tests and Odds ratio were computed using a p value < 0.05 as a level of significance.

Result: A total of 401 pregnant mothers were screened for rubella virus infection. The mean age of the study participants was 26.4 year (SD= 5.4) and the overall sero-prevalence of rubella anti-IgG was 46.4%. In connection, the sero-prevalence of anti-IgM among anti-IgG sero-positive cases was 3.2%. Pregnant women at first trimester (OR=2.49, 95% CI= 1.14-5.42) and HIV sero-status (OR= 0.33, 95% CI= 0.15-0.76) were factors found to be significantly associated with rubella anti-IgG sero-prevalence (p<0.05).

Conclusion: The sero-prevalence of rubella virus infection among pregnant women was considered to be low, showing the high risk of a new infection. In addition to a comprehensive surveillance approach and efforts to determine rubella susceptibility profile among school-aged girls and women of childbearing age, it is also important to consider rubella vaccine in a national vaccination program. [*Ethiop. J. Health Dev.* 2018;32(3):00-000]

Keywords: Rubella virus, unvaccinated, sero-prevalence, pregnant women, risk factors, Ethiopia

Background

Rubella (also called German measles or three-day fever) is among the TORCH infections, which also include Toxoplasmosis, Other (syphilis, varicella-zoster, parvovirus B19), Cytomegalovirus (CMV), and Herpes infections (1-4). These infections are commonly characterized by their easily transmissible nature from mother to fetus during pregnancy and are also responsible for congenital anomalies and possibly stillbirths (3-5). The rubella virus is an enveloped, single stranded, positive sense RNA virus belonging to the Togaviridae family (6). The viral genome encodes for five proteins, three structural proteins (two glycoproteins, E1 and E2 and the capsid protein) and two non-structural proteins (p90 and p150). The E1 glycoprotein contains the antigenic determinants that induce major immune responses and a hemagglutination inhibiting and hemagglutination-neutralizing epitope (7, 8).

Rubella is a contagious disease usually manifested by a mild fever and rash, mostly in young adults and children. However, it can cause a serious birth defect called congenital rubella syndrome (CRS) when a pregnant woman becomes infected, particularly during the first trimester (9-11). The birth defects associated with CRS are ophthalmic, auditory, cardiac, and craniofacial.

Infants with CRS who survive the neonatal period may face serious developmental disabilities and have an increased risk for developmental delay, including autism (5,9,10). Based on a 2015 World Health Organization (WHO) update, globally, over 100,000 babies are born with CRS every year (11). The rate of congenital defects and spontaneous abortion due to rubella virus infection among fetuses can be estimated to 65–85 % when acquired during the first two months of gestation (8). Despite these potentially devastating consequences, many developing countries, including Ethiopia, are yet to include control and prevention of rubella virus infection within their national health strategies (12).

Both humoral and cell-mediated immunity develop following rubella virus infection. Although natural rubella infection generally confers lifelong immunity, rare cases of serologically confirmed re-infections after earlier infection (or immunization) have been reported (13). Immunoglobulin (Ig) G and IgM antibodies are observed about 14-18 days after rubella infection, at about the time when the rash appears. Rubella IgM antibodies wane quickly and are usually undetectable after two months, whereas rubella IgG antibodies persist (14). Serologic tests such as enzyme immunoassay (EIA) that have been calibrated against the WHO

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international standard (RUB-1-94) are important tools to measure rubella specific IgG and IgM, and are considered convenient, sensitive, and accurate methods of screening for rubella virus infection (13,15).

Currently, there is no specific treatment for rubella virus infection. However, the rubella virus infection can be prevented by vaccination which can be available either in monovalent formulation or more commonly in combinations with other vaccines such as with vaccines against measles (MR), measles and mumps (MMR), or measles, mumps and varicella (MMRV). Large-scale rubella vaccination campaigns in the last decade have significantly reduced rubella and associated CRS in many developed nations (11).

In developed countries, the diagnosis of rubella virus is part of prenatal health assessment and also used for understanding the prevalence of the infection, which varies widely in different countries. In sub-Saharan Africa, the sero-positivity of prevalence of rubella virus ranges from 66% to 99% (16). Due to socioeconomic and other factors, pregnant women in developing countries including Ethiopia generally do not undergo routine antibody screening for rubella virus infection (4,17). Although little is known about rubella epidemiology and the incidence of CRS in Africa, it has been estimated that 110,000 CRS cases occur each year during non-epidemic years in developing countries (15).

In Ethiopia, despite measles case-based surveillance system, there is lack of a national screening program for rubella virus infection. Reports showed that rubella is endemic in Ethiopia (12, 18-20). However, there is no documented data regarding the prevalence of rubella virus infection during pregnancy. Furthermore, rubella vaccine is not included in the Ethiopian national immunization programme. Thus, the aim of this study was to assess the sero-prevalence of the rubella virus infection and its associated risk factors among pregnant women who attend antenatal care (ANC) in two hospitals of Amhara Region, Northwest Ethiopia.

Methods

Study setting: This hospital based study was conducted at the antenatal clinics of Debre Markos and Debre Tabor hospitals of Amhara region, Northwest Ethiopia. Debre Markos hospital is the main public hospital in East Gojjam situated in Debre Markos city, the capital of East Gojjam zone. Similarly, Debre Tabor hospital is the main public hospital in the South Gonder zone situated in Debre Tabor city. Both are district hospitals providing different services including antenatal care for pregnant women.

Study design and period: A hospital based cross-sectional study design was employed from March to June 2015.

Study participants: Study participant were recruited from pregnant women who were seeking services at the

antenatal clinics of the two hospitals during the study period and who volunteered to participate in the study. Participants were recruited sequentially until the maximum sample size was achieved. A total of 401 pregnant women - 200 from Debre Markos Hospital and 201 from Debre Tabor Hospital - were included in the study based on the following assumption; 50% prevalence, 5% marginal error, 95% confidence interval and 10% non-response rate. The calculated sample size was equally divided between the hospitals as they represent the wide range of two major districts of the Northwest Ethiopia.

Data collection and laboratory procedures: Interviews were conducted using structured questionnaires to collect information on the socio-demographic characteristics and associated risk factors. Nurses were specifically trained on how to collect the data. About 5ml venous blood sample was collected from all study participants by medical laboratory professionals. Blood samples were centrifuged and serum was separated and stored at -20°C until centrally processed at Bahir Dar Regional Health Research Laboratory Center (BRHRLC) using Enzyme Immuno Assay (EIA) technique (rubella IgG and IgM EIA test kit, Linear Chemicals, Barcelona) according to the manufacturer's instructions.

Rubella sero-positivity was diagnosed by both rubella IgG and IgM Enzyme Immunoassay (EIA) test kits. The Kits are a solid phase enzyme immunoassay based on indirect principle for the qualitative and quantitative detection of IgG and IgM antibodies to rubella in human serum. The microwell plate is coated with rubella antigens. If the specimens contain IgG or IgM antibodies to rubella, they will bind to the antigens coated on the microwell plate to form immobilized antigen-rubella IgG or IgM antibody complexes. Consequently, substrate A and B are added to produce a color that is measured at 4540nm with a microplate reader. The sensitivity and specificity of rubella IgG EIA test kit is 96.4% and >99.0% respectively. Similarly, the sensitivity and specificity of rubella IgM test kit is 90.0% and 94.9% respectively.

Data analysis: Data entry was conducted using Epi Data 3.1 and analysis was performed using SPSS, Version 21 (IBM-SPSS Inc) software. The socio-demographic characteristics of the study participants were summarized by proportions and categorical data were compared by Pearson's Chi-square. Binary and multivariable logistic regression analyses were used to assess factors associated with the rubella virus infection and at 95% confidence interval. Statistical significance was set to P-value < 0.05.

Results

The mean age of study participants was 26.4 (SD= 5.4), with a majority (74.8%) of participants being urban dwellers (Table 1).

Table 1: Socio-demographic characteristics of pregnant women from Debre Markos and Debre Tabor hospitals of Northwest Ethiopia, 2015.

Characteristics		Total (%)	Positive for anti-rubella IgG	Negative for anti-rubella IgG	P value [#]
Age in years	≤ 20	52 (13.0)	28 (53.8)	24 (46.2)	0.350
	21-30	281 (70.1)	124 (44.1)	157 (55.9)	
	≥31	68 (16.9)	34 (50.0)	34 (50.0)	
Residence	Urban	300 (74.8)	134 (44.7)	166 (55.3)	0.235
	Rural	101 (25.2)	52 (51.5)	49 (48.5)	
Marital status	Married	397 (99.0)	183 (46.1)	214 (53.9)	0.249
	No married	4 (1.0)	3 (75.0)	1 (15.0)	
Educational status	No formal education	81 (20.3)	35 (43.2)	46 (56.8)	0.529
	Primary school	80 (20.0)	42 (52.5)	38 (47.5)	
	High school	103 (25.8)	50 (48.5)	53 (51.5)	
	College or university	136 (34.0)	59 (43.4)	77 (56.6)	
Occupational status	Employee	96 (23.9)	35 (36.5)	61 (63.5)	0.083
	House wife	168 (41.9)	80 (47.6)	88 (52.4)	
	Merchant	60 (15.0)	28 (46.7)	32 (53.3)	
	No job	77 (19.2)	43 (55.8)	34 (44.2)	
Monthly income	≤1000	128 (34.1)	59 (46.1)	69 (53.9)	0.700
	1001-3000	154 (41.1)	74 (48.1)	80 (51.9)	
	3001-5000	66 (17.6)	26 (39.4)	40 (60.6)	
	>5001	27 (7.2)	12 (44.4)	15 (55.6)	

[#] P value of Chi-square test

Based on EIA for rubella virus the overall sero-prevalence of rubella anti-IgG was 46.4% and the sero-prevalence of anti-IgM within IgG sero-positive was 3.2%. Although it was not statistically significant, the IgG sero-prevalence of rubella virus was relatively higher among pregnant women from Debre Tabor hospital (Table 2).

Table 2: Sero-prevalence of rubella virus among pregnant women from Debre Markos and Debre Tabor hospitals of North West Ethiopia.

Character	Total		Debre Markos Hospital		Debre Tabor Hospital	
	Positive N (%)	Negative N (%)	Positive N (%)	Negative N (%)	Positive N (%)	Negative N (%)
Anti-Rubella IgG	186 (46.4)	215 (53.6)	87 (43.5)	113 (56.5)	99 (49.3)	102 (50.7)
Anti-Rubella IgM*	6 (3.2)	179 (96.8)	3 (3.4)	84 (96.6)	3 (3.0)	96 (97.0)

*Within Rubella IgG positive, IgG= immunoglobuline G, IgM= immunoglobuline M

In Table 3 below, the bivariate logistic regression analysis shows that stage of pregnancy (OR=2.49, 95% CI= 1.14-5.42) and HIV-sero status (OR= 0.33, 95% CI= 0.15-0.76) were significantly associated with rubella virus sero-positivity. Pregnant women who were on their first trimester were about three times more likely to be positive for anti-rubella IgG compared to those women in their third trimester (AOR= 3.16, 95% CI= 1.36-7.36). Similarly, those pregnant women with HIV infection were found to be about five times

(AOR=5.14, 95% CI= 1.94-13.57) more likely to be positive for rubella virus infection (Table 3). However, the association between rubella virus sero-positivity and other factors like gravidity, source of drinking water, history of spontaneous abortion, history of neonatal death, history of premature delivery, alcohol consumption, and history of reproductive tract infection and history of other chronic diseases other than HIV/AIDS were found to be statistically insignificant ($p>0.05$).

Table 3: **Bivariate and multivariate analysis for the risk factors associated with rubella virus infection among pregnant women in Debre Markos and Debre Tabor hospitals.**

Characteristics		Total (%)	Rubella positive N (%)	Crude Odds Ratio (95% CI)	P value	Adjusted OR (95 % CI)	P value
Time of pregnancy	1 st trimester	32(8.3)	21(11.9)	2.49[1.14-5.42]	0.022	3.16[1.36-7.36]	0.027
	2 nd trimester	140(36.5)	64(36.2)	1.09[0.71-1.68]	0.668	1.23[0.77-1.94]	
	3 rd trimester	212(55.2)	92(52.0)	1		1	
Gravidity	Prim-gravidae	164(41.2)	80(43.5)	1	0.393	-	-
	Multi-gravidae	234(58.3)	104(56.5)	0.84[0.56-1.25]		-	-
Source of drinking water	Pipe	316(79.0)	148(80.0)	1	0.649	-	-
	Other sources*	84(21.1)	37(19.0)	0.89[0.55-1.45]		-	-
HIV sero-status	Negative	349(87.5)	167(90.3)	1	0.009	1	0.001
	Positive	16(4.0)	10(5.4)	1.82[0.65-5.11]		5.14[1.94-13.57]	
	Not known	34(8.5)	8(4.3)	0.33[0.15-0.76]		-	-
History of spontaneous abortion	No	347(86.5)	158(85.9)	1	0.387	-	-
	Yes	54(13.5)	28(15.1)	1.29[0.73-2.28]		-	-
History of neonatal death	No	368(92.0)	170(91.9)	1	0.941	-	-
	Yes	32(8.0)	15(8.1)	1.03[0.49-2.12]		-	-
History of premature delivery	No	385(96.7)	178(96.7)	1	0.995	-	-
	Yes	13(3.3)	6(3.3)	0.99[0.33-3.02]		-	-
Alcohol consumption	No	298(74.3)	137(73.7)	1	0.779	-	-
	Yes	103(25.7)	49(26.3)	1.06[0.68-1.67]		-	-
History of other chronic diseases**	No	392(97.8)	183(98.4)	1	0.433	-	-
	Yes	9(2.2)	3(1.6)	0.57[0.14-2.32]		-	-

*Includes: spring and river ** Includes chronic diseases other than HIV/AIDS

Discussion

Due to the absence of vaccination programs CRS is more common in developing countries compared to developed nations. The incidence of rubella virus among the 17-22 age group was estimated to be 80-88% in less developed part of the world. Consequently, 12-20% of these populations are still susceptible to infection with the rubella virus (21).

This study assessed the sero-prevalence of rubella virus and its associated risk factors among pregnant women in Northwest Ethiopia. The results showed that the sero-prevalence of anti-rubella virus IgG was 46.4%. This is significantly lower than the previous study conducted in Ethiopia among young adult females (88% to 94%) (19) in major cities of the country. The sero-prevalence of rubella virus appears to vary among different communities by geographic locations based on their vaccination status and occurrences of epidemic. Similar studies from other non-vaccinated African countries reported higher IgG sero-prevalence among pregnant women, for example in Benin (94.0%) (23), in Burkina Faso (77.0%, 95.0%) (17,24), in Egypt (87.3%) (22) and in Sudan (95.1%) (25) which were notably higher prevalence compared to the findings in this study.

Though there is substantial transmission of rubella virus in the study area, our results show that the majority of the study participants (53.6%) were not immune against rubella virus. The lower sero-prevalence of anti-rubella IgG in this study might be linked with the absence of rubella vaccination in the national vaccination campaign. As there is no surveillance programme of rubella virus infection and CRS in the country, it is important to determine the risk and level of CRS to determine the need for introduction of rubella vaccination especially during early in life.

Though it is not statistically significant, our study reported that there was an increased proportion of anti-rubella IgG sero-positivity as the age of pregnant mothers' increases. This finding is found to be consistent with the study reported from Nigeria and Burkina Faso (17,26). The relationship between HIV and rubella virus is poorly understood. In our study HIV sero-status was found to be significantly associated with rubella sero-positivity. Thus, further study with strong statistical power and longitudinal design is recommended to understand the relationship between these two infections.

A potential limitation of this study is the temporal relationship that may be interpreted because of its cross-sectional design. The use of nurses in the ANC as data collectors may have also introduced desirability bias. The absence of association between various risk factors and rubella virus might be because the study did not have strong statistical power to detect the differences. This study overlooked the importance of including clinical data, outcome of the pregnancy and the sero-positivity of

the newly born babies which would have been a good opportunity to describe the clinical presentation and the consequences of rubella virus infection during pregnancy on the newborn.

Conclusion and Recommendation:

In this study, the rubella virus sero-prevalence was found to be low as compared to other similar studies. On the contrary, susceptibility to rubella virus infection is believed to be very high in the study areas the pregnant women were all unvaccinated. In order to better understand the burden and epidemiology of rubella and CRS, the Ethiopian Ministry of Health may consider adopting a comprehensive approach to surveillance, establishing sentinel surveillance for CRS, and conducting appropriate studies to assist in defining the rubella susceptibility profile in school-aged girls and women of childbearing age. Information from such studies will be useful in the consideration of appropriate rubella control strategies in Ethiopia.

Abbreviations

ANC: Antenatal Care; BRHRLC: Bahir Dar Regional Health Research Laboratory Center; CMV: Cytomegalovirus; CRS: Congenital rubella syndrome; EIA: Enzyme Immuno Assay; HIV/AIDS: Human Immunodeficiency Syndrome; IgG: Immunoglobulin G; IgM: Immunoglobulin M; TORCH: Toxoplasmosis, Other (syphilis, varicella-zoster, parvovirus B19).

Ethical Statement

Ethical clearance was obtained from Bahir Dar University College of Medicine and Health Science research ethics review committee. Permission was obtained from Amhara Regional State Health Office. Additional permission was obtained from DM and DT hospitals. The purpose of the study was explained to the participants and informed written consent was obtained from all study participants prior to data collection. To keep confidentiality of the participants, personal identifiers were not included in the data collection, anonymity was ensured throughout the research process, and the information was utilized only for research purposes. Participation was entirely voluntary.

Competing interests

The authors declare no conflict of interests.

Author's contributions

BT, DM, MG were involved in the conceptualization and design of the study. BT, DM, YZ and EA involved in conducting of the research. BT, DM, YZ, EA carried out the data analysis and interpretation of the data. BT drafted the manuscript; all authors read and approved the final submitted version.

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References

1. Stegmann BJ, Carey JC. TORCH infections. Toxoplasmosis, other (syphilis, varicella zoster, parvovirus B19), Rubella, Cytomegalovirus (CMV), and Herpes infections. *Curr Womens Health Rep.* 2002;2:253-8.
2. Tubadkar D, Mathrur M, Rele M. Seroprevalence of TORCH infection in bad obstetric history. *Indian J Med Microbiol.* 2003;21:108-10.
3. Sen MR, Shukla BN, Tuhina B. Prevalence of Serum Antibodies to TORCH Infection in and Around Varanasi, Northern India. *J Clinical Diagnostic Research.* 2012;6(9):1483-1485.
4. Li Z, Yan C, Liu P, Yan R, Feng Z. The prevalence of the serum antibodies to TORCH among women before pregnancy or in the early period of pregnancy in Beijing. *Clinica Chimica Acta.* 2009; 403: 212-15.
5. Ishaque S, Yakoob MY, Imdad A, Goldenberg RL, Eisele TP, Bhutta ZA. Effectiveness of interventions to screen and manage infections during pregnancy on reducing stillbirths: a review. *BMC Public Health.* 2011;11(3):S3
6. Frey TK. Molecular Biology of Rubella Virus. *Advance Virus Res.* 1994;44:69-160.
7. Chaye H, Chong P, Triplet B, Brush B, Gillam S. Localization of the Virus Neutralizing and Hemagglutinin Epitopes of El Glycoprotein of Rubella Virus. *Virol.* 1992;189:483-492.
8. Lee JY, Bowden DS. Rubella Virus Replication and Links to Teratogenicity. *Clin Microbiol Rev.* 2000;13(4): 571-587.
9. Binnicker MJ, Jespersen DJ, and Harring JA. Multiplex Detection of IgM and IgG Class Antibodies to *Toxoplasma gondii*, Rubella Virus, and Cytomegalovirus using a Novel Multiplex Flow Immunoassay. *Clin Vaccine Immunol.* 2010, 17(11):1734-1738.
10. World Health Organization. Rubella vaccines. WHO position paper. *Wkly Epidemiol Rec.* 2000;75:161-72.
11. WHO. Rubella fact sheet. World Health Organization, Updated March 2017. Available at: <http://www.who.int/mediacentre/factsheets/fs367/en/>
12. Mitiku K, Bedada T, Masresha B, Kegne W, Nafotraore F, *et al.* The Epidemiology of Rubella Disease in Ethiopia: Data from the Measles Case-Based Surveillance System. *Infect Dis.* 2011;204:239-42. (17)
13. Robertson, SE, Cutts FT, Samuel R, and Diaz-Ortega JL. Control of rubella and congenital rubella syndrome (CRS) in developing countries. Part 2. Vaccination against rubella. *Bull. W. H. O.* 1997;75:69-80.
14. World Health Organization Department of Immunization, Vaccines and Biologicals. Manual for the laboratory diagnosis of measles and rubella virus infection. Second edition. August 2007. Geneva 27, Switzerland. WHO/IVB/07.01. www.who.int/vaccines-documents/
15. Dimech W, Grangeot-Keros L, Vauloup-Fellous C. Standardization of assays that detect anti-rubella virus IgG antibodies. *Clin Microbiol Rev.* 2016;29:163-174. doi:10.1128/CMR.00045-15.
16. Goodson JL, Masresha B, Dosseh A, Byabamazima C, Nshimirimana D, Cochi S, Reef S. Rubella Epidemiology in Africa in the Pre-vaccine Era, 2002–2009. *J Infect Dis.* 2011;204:S215–S225.
17. Linguissi LSG, Nagalo BM, Bisseye C, Kagoné TS, Sanou M, *et al.* Seroprevalence of toxoplasmosis and rubella in pregnant women attending antenatal private clinic at Ouagadougou, Burkina Faso. [Asian Pac J Trop Med](http://AsianPacJTropMed). 2012;5(10):810-3. doi: 10.1016/S1995-7645(12)60148-5.
18. Cutts FT, Vynnycky E. Modelling the incidence of congenital rubella syndrome in developing countries. *Int J Epidemiol.* 1999;28:1176–84.
19. Gebreselassie L and Abebe A. The immune status of young adult females in Ethiopia to rubella virus infection. *Bull WorldHealth Organ.* 1985; 63(5):927–30.
20. Cutts FT, Abebe A, Messele T, Dejene A, Enquesselassie F, *et al.* Sero-epidemiology of rubella in the urban population of Addis Ababa, Ethiopia. *Epidemiol Infect.* 2000;124:467-79.
21. Vauloup-Fellous C, and Grangeot-Keros L. Immune response after primary rubella virus infection and after vaccine. *Clin Vaccine Immunol.* 2007;14:644-647.
22. Al-Sheref F, Jefri OH and El-Sayed ZMF. Seroprevalence of Rubella among Pregnant Women and Young Females. *Egypt J Med Microbiol.* 2010;19 (1):119-28.
23. Paschale M, Ceriani C, Cerulli T, Cagnin D, Cavallari S, *et al.* Antenatal screening for *Toxoplasma gondii*, Cytomegalovirus, rubella and *Treponema pallidum* infections in northern Benin. *Trop Med Int Health.* doi:10.1111/tmi.12296.
24. Tahita MC, Hübschen JM, Tarnagda Z, Ernest D, Charpentier E, *et al.* Rubella sero-prevalence among pregnant women in Burkina Faso. *BMC Infect Dis.* 2013;13:164.
25. Adam O, Makkawi T, Kannan A and Osman ME. Sero-prevalence of rubella among pregnant women in Khartoum state, Sudan. *East Mediterr Health J.* 2013; 19(9).
26. Bukbuk DN, el Nafaty AV, Obed JY. Prevalence of rubella specific IgG antibody in non-immunized pregnant women in Maiduguri North Eastern Nigeria. *Cent Eur J Public Health.* 2002;10 (1-2): 21-23.