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Ph.D. Thesis

Cervical Cancer, Risk Factors and Feasibility of Visual Inspection with Acetic Acid Screening Method in Khartoum State, Sudan

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Contents

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Abstract

Background: Cervical cancer is one of the leading causes of death for middle-aged women in the developing world, yet it is almost completely preventable if precancerous lesions are identified and treated in a timely manner. There are different methods for control and prevention of cervical cancer which include conventional cytology (Pap smear), liquid-based cytology, human papillomavirus (HPV) screening, and vaccination against HPV. Cytology-based and HPV screening method are hard to be implemented in developing countries. Therefore there is an increased interest in the use of visual screening by use of acetic acid (VIA) test to identify cervical cancer in developing countries.

The general aim of the study was to determine feasibility and acceptability of VIA screening method in a primary health centers in Khartoum, Sudan. The specific aims were: (i) to study risk factors of VIA positivity; (ii) to compare performance of VIA and Pap smear test in detection of cervical cancer;(iii) to investigate predictors of cervical cancer being at advanced stage at diagnosis; and (iv) to assess knowledge and practice of physicians on cervical cancer screening in Sudan.

Materials and Methods: Descriptive cross-sectional surveys were conducted in four consecutive phases during the study period. In the first phase a pilot study was undertaken to study cervical cancer risk factors. At the same time data were collected from the cancer registry unit to determine predictors of different stages of cervical cancer at diagnosis. In the second phase a definitive study was carried out to determine the performance of VIA test compared to Pap smear, and in the third phase a survey was conducted to assess knowledge and practice of physicians about cervical cancer screening. Data were collected from the target study populations by using different methods**:** semi- structured questionnaire inquiring demographic, reproductive factors and other risk factors. VIA and conventional Pap smear methods were used to screen the participating women, followed by colposcopy and biopsy for confirmation of the positive results. Further, a self–administered questionnaire was used to collect data from physicians about their knowledge and practices of cervical cancer screening. Completed data of diagnosed women with cervical cancer in year 2007 were obtained from the cancer registry unit

at Radiation and Isotopes Center in Khartoum and analyzed to determine predictors of different stages of cervical cancer at diagnosis.

Data were analyzed by STATA Version 9.2 Stata Corp, Texas USA**.** Descriptive statistics, *t*-test and Chi square test were used to detect any significant difference between continuous and categorical variables. Performance of VIA and Pap smear tests was assessed by sensitivity, specificity, positive and negative predictive values. The relationship between predictor variables and cervical cancer stages at diagnosis was then examined by logistic regression. *P* value, odds ratio (OR) and 95% confidence interval (CI) were reported.

Results: In the pilot study asymptomatic women (n=100) were recruited for the study and screened for cervical cancer by VIA test. The study revealed that 16% of screened women had VIA positive test result. Statistically significant associations were observed between being positive with VIA test and the following variables: uterine cervix laceration (OR18.6; 95% CI: 4.64–74.8), assisted vaginal delivery (OR 13.2; 95% CI: 2.95–54.9), parity (OR 5.78; 95% CI: 1.41–23.7), female genital mutilation (OR 4.78; 95% CI: 1.13–20.1), and episiotomy (OR 5.25; 95% CI: 1.15–23.8).

Data of 197 women diagnosed with different stages of cervical cancer showed that there was an association between older age and advanced stage at diagnosis of cervical cancer (OR1.03, 95% CI: 1.01–1.05), African ethnicity (OR 1.76, 95% CI: 1.01–3.05), living in a rural area (OR: 1.13, 95% CI: 1.78–5.50). In addition, being uninsured was associated with an almost eight-fold increased odds (OR: 7.7, 95% CI: 3.76–15.4).

In the definitive study of a large sample size (n=1250) asymptomatic women living in the study area during the year 2009-2010 took part. The recruitment and response rates were high 79% (985/1250) and 95% (934/985), respectively. All eligible women were screened by VIA and Pap smear followed by colposcopy and biopsy for positive cases. The tests identified altogether12.7% (119/934) positive women, VIA significantly more than Pap smear (7.6% versus 5.1%; *p*=0.004). There was an overlap between VIA and Pap smear in positive results of 20.2% (24/119) of all positive women. Colposcopy and biopsy of positive women confirmed that 73.9% (88/119) were positive for intraepithelial cervical neoplasia (CIN). VIA had higher sensitivity than Pap smear (57.7% versus 30.8%) respectively. Out of 88 confirmed positive cases, 25% (22/88) cases were invasive cervical cancer in stage 1, of which 19(21.6%) versus 3(3.4%); *p*=0.001) were detected by VIA and Pap smear respectively. Parallel result of

sensitivity and specificity of VIA and Pap test was 69.4 % 95% respectively. The parallel tests have high sensitivity compared to each test individually, but parallel specificity of both tests is lower compared to specificity of each test independently.

A cross–sectional survey of physicians (n=230) revealed that 83% of physicians perceived cervical cancer as a major health problem in Sudan, and 62% of all physicians stated that this cancer can be tackled by diagnosis and treatment, 80% identified that the solution can be by initiation of cervical cancer screening program, while 43% claimed that this cancer can be prevented by vaccination of the women against HPV.

Conclusion: The study findings showed that women who had uterine cervix laceration, assisted vaginal delivery, female genital mutilation, or episiotomy were more at risk for being VIA positive. The result of screening revealed that VIA had higher sensitivity than Pap smear. VIA is useful, feasible and acceptable cervical cancer screening method in a primary health care setting in Khartoum State in Sudan for screening of cervical cancer, but positive results need to be confirmed by colposcopy and biopsy. Women with cervical cancer who are elderly, not covered by health insurance, who are of African ethnicity, and living in a rural area, are more likely to be diagnosed at an advanced stage of cervical cancer in Sudan. These women should be targeted for cervical cancer screening and to have health insurance. Future implementation of cervical cancer screening programme can benefit from the adequate knowledge and practice of physicians on cervical cancer. More efforts are needed to develop strategies for promotion of cancer prevention methods in continuous medical education.

Keywords: Cervical cancer, risk factors, VIA, Pap smear, screening, feasibility, Khartoum, Sudan

Abbreviations

ASIR	Age standardized incidence rate
CFR	Case fatality rate
CI	Confidence Interval
CIN	Cervical intraepithelial neoplasm
DNA	Deoxyribonucleic acid
DVI	Direct visual inspection
EMRO	Eastern Mediterranean Regional Office
FIGO	International Federation of Obstetrics and Gynecology
IARC	International Agency on Research for Cancer
HLA	Human lymphocyte antigen
HPV	Human Papillomavirus
OR	Odd ratio
RICK	Radiation and Isotopes Center in Khartoum
RNA	Ribonucleic acid
Pap	Papanicolaou
PPV	Positive Predictive Value
NPP	Negative Predictive Value
SCJ	Squamo-columnar junction
VIA	Visual inspection with used of acetic acid
VIAM	Visual inspection with use of acetic acid with low-level magnification
WHO	World Health Organization

List of Orignal Publications

1. Ahmed Ibrahim; Vibeke Rasch; Eero Pukkala; Arja R Aro. Cervical cancer risk factors and feasibility of visual inspection with acetic acid screening in Sudan. International Journal of Women's Health 2011, 3:117-122.
2. Ahmed Ibrahim; Vibeke Rasch; Eero Pukkala; Arja R Aro. Predictors of cervical cancer being at an advanced stage at diagnosis in Sudan. International Journal of Women's Health 2011; 3:385-389.
3. Ahmed Ibrahim; Eero Pukkala; Rasch V; Arja R Aro. Cervical cancer screening in primary health care setting in Sudan: a comparative study of visual inspection with acetic acid and Pap smear. International Journal of Women's Health 2012; 4:67-73.
4. Ahmed Ibrahim; Vibeke Rasch; Eero Pukkala; Arja R Aro. Physicians' knowledge and practice of cervical cancer screening in Khartoum, State, Sudan.*(submitted)*

N.B: The original publications are included with the permission of the publisher (Appendix 11.7)

1. Background

1.1. Global Burden of Cervical Cancer

Cervical cancer is the most common cancer in women in sub-Saharan Africa and is a leading cause of death in women in Southern Africa. The disease is a prime example of global inequality in health. Mortality from cervical cancer in developed countries is substantially lower than in developing nations because of the availability of prevention, early detection, and treatment.[1] Cervical cancer is the third most common cancer in women, and the seventh overall, with an estimated 530 000 new cases in 2008. More than 85% of the global burden of cervical cancer occurs in developing countries, where it accounts for 13% of all female cancers. High-risk regions are Eastern and Western Africa (ASR greater than 30 per 100,000), Southern Africa (26.8 per 100,000), South-Central Asia (24.6 per 100,000), South America and Middle Africa (ASRs 23.9 and 23.0 per 100,000 respectively). Rates are lowest in Western Asia, Northern America and Australia/New Zealand (ASRs less than 6 per 100, 00). Cervical cancer remains the most common cancer in women only in Eastern Africa, South-Central Asia and Melanesia.[2]

Overall, a proportional prevalence rate of cervical cancer was 52%, and cervical cancer was responsible for 275,000 deaths in 2008, about 88% of which occur in developing countries: 53,000 in Africa, 31 700 in Latin America and the Caribbean, and 159.800 in Asia.[2] Table1shows cases, deaths and 5-year prevalence of cervical cancer by regions.[2] According to the recent data, approximately 85% of new cases of cervical cancer occur in developing countries.[3] Approximately 80%-90% cervical cancer cases in developing countries occur among women age 35 and older. Cervical cancer progresses slowly from precancerous lesion to advanced cancer. Globally the incidence of the cancer is very low in women under age of

25 years. However, the incidence increases at age of 35 to 40 years and reaches the maximum in women in their 50s and 60s.[4]

Table 1: Estimated cases, deaths and 5-year prevalence of cervical cancer [2]

Estimated numbers (thousands)	Cases	%	Deaths	%	5-year prevalence	%
More developed regions	**76**	**14.3**	**32**	**11.6**	**266**	**17.1**
Less developed regions	**453**	**85.5**	**242**	**88.0**	**1288**	**82.8**
WHO Africa region (AFRO)	75	14.2	50	18.2	194	12.5
WHO America region (PAHO)	80	15.1	36	13.1	270	17.4
WHO East Mediterranean region(EMRO)	18	3.4	11	4.0	52	3.3
WHO Europe region (EURO)	61	11.5	28	10.2	206	13.2
WHO South-East Asia region (SEARO)	188	35.5	102	37.1	498	32.0
WHO Western Pacific region (WPRO)	105	19.8	46	16.7	332	21.4
IARC membership (22 countries)	193	36.4	96	34.9	546	35.1
United States of America	11	2.1	3	1.1	40	2.6
China	75	14.2	33	12.0	232	14.9
India	134	25.3	72	26.2	338	21.7
European Union (EU-27)	31	5.8	13	4.7	106	6.8
World	**530**	**100**	**275**	**100**	**1555**	**100**

This table is Adapted from Globocan2008 [2] with some modifications

The cumulative risk of developing risk of developing cervical cancer throughout the life reflects high risk in developing countries and low in developed countries and reveals a high worldwide discrepancy (Figure 1). Generally the risk estimates correlation with the existing organized screening programs. The lifetime risk of developing cervical cancer was observed to be low in more developed countries.[2]

1.2. Burden of cervical cancer in Africa

A lack of precise information about cancer magnitude in the Africa due to limited cancer registries masks the picture of the cancer problem in this continent. Therefore the burden of disease is frequency estimated based on average data from neighboring countries (Figure1).Cervical cancer is the major cancer among the women in Africa followed by breast cancer.

Fig.1: Cumulative risk of developing cervical cancer by regions, age 0-60 years [2]

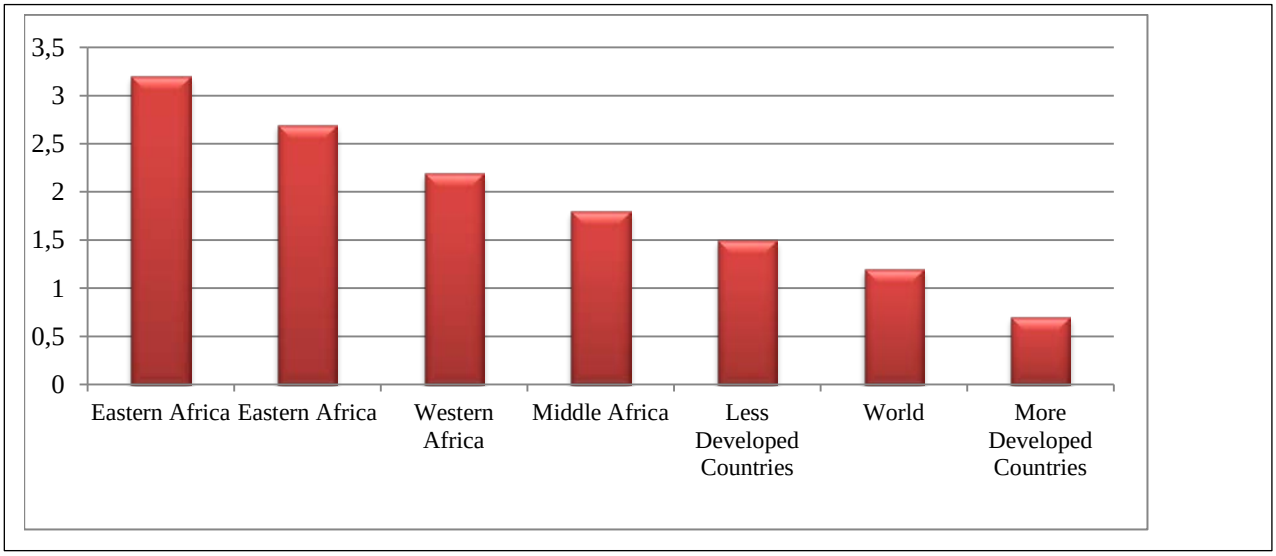


Table 2: Cases, deaths and case fatality rate of cervical cancer in Africa by region [2]

Region	Cases	%	Deaths	%	CFR
Eastern Africa Region	33,903	43.0	27,147	44.0	80.1
Northern Africa Region	8201	10.4	6588	10.7	80.3
Middle Africa Region	8201	10.4	6687	10.8	81.5
Western Africa Region	20,919	26.5	16,793	27.2	80.3
Southern Africa Region	7698	9.8	4455	7.2	57.9
Africa	78,922	100	61,670	100	78.1

Table3: Cervical cancer: cases, deaths and case fatality rate (CFR) by region and country [2]

Region/Country	**Cases**	**Deaths**	**CFR**
Eastern Africa Region	**33,903**	**27,147**	**80.1**
Burundi	899	722	80.3
Comoros	97	79	81.4
Djibouti	113	90	79.6
Eritrea	548	438	79.9
Ethiopia	7,619	6,081	79.8
Kenya	2,619	2,111	80.6
Madagascar	2,238	1,795	80.2
Malawi	1,766	1,405	79.6
Mauritius	111	61	55.0
Mozambique	2,058	1,654	80.4
Rwanda	1,087	878	80.8
Somalia	1,134	906	79.9
Tanzania	7,515	6,009	80.0
Uganda*	2,429	1,932	79.5

Zambia	1,650	1,340	81.2
Zimbabwe*	1,817	1,492	82.1
Northern Africa Region	**8,201**	**,588**	**80.3**
Algeria*	1,726	1,391	80.6
Egypt*	2,713	2,178	80.3
Libya	218	175	80.3
Morocco	1,550	1,247	80.5
Sudan	***1,664***	***1,354***	***81.4***
Tunisia*	284	229	80.6
Middle Africa Region	**8,201**	**6,687**	**81.5**
Angola	1,158	926	80.0
Cameroon	1,759	1,419	80.7
Central Africa Republic	374	306	81.8
Chad	681	555	81.5
Congo Brazzaville*	303	242	79.9
Congo	3,709	3,058	82.4
Equatorial Guinea	45	37	82.2
Gabon	164	135	82.3
Southern Africa Region	**7,698**	**4,455**	**57.9**
Botswana	156	126	80.8
Lesotho	479	391	81.6
Namibia	133	109	82.0
Southern Africa Republic	6,742	3,681	54.6
Swaziland	186	150	80.6
Western Africa Region	**20,919**	**16,793**	**80.3**
Benin	561	448	79.9
Burkina Faso	921	724	78.6
Cape Verde	47	38	80.9
Cote d'Ivoire	1,497	1,192	79.6
Gambia	157	24	79.0
Ghana	1,958	1,572	80.3
Guinea Bissau	124	99	79.8
Guinea	1,444	1,138	78.8
Liberia	320	256	80.0
Mali*	1,336	1,076	80.5
Mauritania	259	209	80.7
Nigeria	9,922	8,030	80.9
Niger	679	532	78.4
Senegal	804	640	79.6
Sierra Leone	452	362	80.1
Togo	435	349	80.2

*Country has a population-based cancer registry

Table 2 demonstrates cases, deaths and case fatality rate of cervical cancer in African regions. The highest case load of cervical cancer is in the eastern Africa region (43%) and lowest in the western Africa region (9.8%). The frequency of cervical cancer death rate is also proportionally higher in the eastern Africa region and lower in the western Africa region; 44% and 7.2% respectively. Table 3 shows cervical cancer cases, deaths and case fatality rate by region and country. In this table approximately there are equal proportions of CFR across all African regions and countries (79%-81%) except in the southern African region and the Southern African Republic, where CFR is the lowest 57.9% and 54.6% respectively.

1.3. Etiology and risk factors

The major risk factor for cervical cancer is infection with human papillomavirus (HPV). The most common HPV types in patients, in descending order of frequency, were types 16, 18, 45, 31, 33, 52, 58, and 35. Munoz et al [5] wrote in their abstract: For studies using the GP5+/6+ primer, it found that the pooled odds ratio for cervical cancer associated with the presence of any HPV was 158.2 (95 % confidence interval, 113.4 to 220.6). The odds ratios were over 45 for the most common and least common HPV types. Fifteen HPV types were classified as high-risk types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82); three were classified as probable high-risk types (26, 53, and 66); and 12 were classified as low-risk types (6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, and CP6108). There was good agreement between the epidemiological classification and the classification based on phylogenetic grouping. The infection with HPV genotypes has geographical variation. In the United States, HPV-16 was the most common type, but HPV-58 was the second most prevalent in Mexican population.[6] In Spain 75% of screened women was sero-positive for HPV-58.[7] HPV-58 was the second most common genotype in Japan.[8] In China the prevalence of HPV-58 was 24%.[9] In Paraguay, HPV-58 was detected in 2.7% of cervical carcinomas. [10] In Brazil prevalence rates of HPV-16, HPV-58, HPV-31 and HPV-18 were 49%, 13, 12% and 4.5% respectively.[11] The association between cervical cancer and high risk oncogenic types of HPV is clearly demonstrated.[12] Women with cervical intraepithelial neoplasia III and invasive cervical cancer have high prevalence of HPV-16. HPV-18.[13] Lee- Wen et al reported that HPV-18 was present more frequently in (84.6%) adenocarcinomas and adenosquamous carcinomas.[14] In Mali, HPV DNA was identified in 97% of cervical cancer cases, and HPV types 16, 18, and 31 were detected in 60% of cases and 45% of controls.[15] Cervical cancer risk is significantly associated with multi-parity. It was 5.1 fold for women with 14 pregnancies or more. [16] In Mali, risk factor of cervical cancer for parity of

>10 was 4.8 fold compared to parity of <5 children.[15] Increased number of pregnancies and younger age of having the first child is significantly associated with the risk of cervical cancer.[16] Infertility, intrauterine device use and vaginal deliveries were associated with cervical intraepithelial neoplasia in American Indian women.[17] Long duration smoking (20 or more years) was associated with a two-fold increase in the risk of squamous cell carcinoma, but smoking was not associated with the risk of adenocarcinoma.[18]Long-term use of oral contraceptives could be a co-factor that increases risk of cervical carcinoma by up to four-fold in women who are positive for cervical HPV.[19] Cervical cancer incidence rates have been observed to vary between different socio-economic groups, and the importance of these factors may vary between different geographical regions.[20]

There is also genetic risk for cervical cancer; evidence revealed that familial clustering of cervical cancer and its precursor forms.[21-22] Genetic susceptibility to cervical cancer is related to HLA class II. HLA, B7 and DQB1 are positively associated with cervical neoplasia while DRB1 is negatively associated with disease.[23]

1.4. Preventive factors

Use of barrier methods of contraception is associated with a reduced the risk of cervical cancer.[24] Males' circumcision is associated with a reduced risk of penile HPV infection among males and reduced risk of cervical cancer among their female partners.[25] Vaginal spermicidal are effective in preventing cervical cancer, which may be due to antiviral action.[26] A systematic review of evidence showed a possible protective factor of diet that contains fruits, vegetables, and some of bioactive components such as vitamins C and E, and the carotenoids. Its protective effect is against HPV persistence.[27]

1.5. Pathogenesis of cervical cancer

The normal cervix is covered on its outer surface by a non-keratinizing, stratified squamous epithelium, which is continuous below with the squamous epithelium lining the vagina, and above abuts onto the mucus secreting columnar epithelium lining the endocervical canal and its associated crypts. The junction between the two epithelia normally coincides with the external os, but this is not a constant relation. At puberty, in pregnancy and in some steroid contraceptive users, changes in the size and shape of the cervix result in the squamo-columnar junction (SCJ) being carried out on to the anatomical ectocervix.[28]

There are two primary histologic abnormalities accounting for the majority of cervical cancer, squamous cell carcinoma (SCC) and adenocarcinoma. The majority of cervical cancer cases (>70%) are SCC, which is thought to arise from the transformation zone of the cervix.[29, 30]

SCC develops from the transformation zone, which locates at the junction between the squamous and columnar cells of the cervix (squamo-columnar junction), which migrates from the exocervix to the distal endocervical canal with advancing age.[31] The second type of cervical cancer is adenocarcinoma, which develops from the mucus-producing cells of the endocervix, accounts for approximately 18 percent of cervical carcinomas. The remainders of cervical carcinomas are adenosquamous (4%) and other carcinomas (5%) or malignancies (1.5%).[31]

The primary precancerous lesion is known cervical intraepithelial neoplasm (CIN) and it is classified into three types: CIN1 corresponds to mild dysplasia, CIN2 to moderate dysplasia, and CIN3 which includes severe dysplasia, carcinoma in situ and invasive carcinomas develop. Bethesda system is designed to provide simplification of cytological diagnoses. In this system, lesions with CIN1 are classified as low-grade squamous intraepithelial lesions

(LSIL) and lesions with CIN2 or CIN3 are combined as high-grade squamous intraepithelial lesions.[32, 33, 34]

The natural history of CIN indicates that cervical cancer does not develop suddenly and is preceded by precancerous changes of the cervix; this was reported in several studies. Holowaty et al [35] reported that both mild and moderate dysplasia were more likely to regress than to progress. The risk of progression from mild to severe dysplasia or worse was only 1% per year, but the risk of progression from moderate dysplasia was 16% within 2 years and 25% within 5 years. Most of the excess risk of cervical cancer for severe and moderate dysplasia occurred within 2 years of the initial dysplastic smear. After 2 years, in comparison with mild dysplasia, the relative risks for progression from severe or moderate dysplasia to cervical cancer in situ or worse was 4.2 and 2.5 respectively.[31] In another study by McCredie et al reported that the rate of progression of CIN3 to cancer was estimated as 31.3 percent in 30 years. This rate was determined using retrospective data from clinical study in New Zealand between 1965 and 1974 that left a number of women with CIN3 disease incompletely treated or untreated.[36]

It is also recognized that higher grade lesions of CIN 2 and CIN 3 are more likely to progress to invasive carcinoma and are usually treated without unjustified delay.[5] When cervical cancer is detected by screening in early micro- invasive cervical cancer stage and confirmed by directed excision biopsy, such a finding has a low risk of metastatic disease and therefore it can be easily treated with a good outcome. If cervical cancer is diagnosed in advanced stages, treatment in such cases is very difficult with very poor outcome .[37, 38, 39]

2. Prevention and control of cervical cancer

Different methods of cervical cancer prevention of control have been developed and implemented worldwide. These methods include early diagnosis and treatment of

precancerous lesions has led to a significant reduction in the burden of the disease. Screening for precancerous lesions can be done in several ways including, cervical cytology (Pap smear), and visual inspection of the cervix with acetic acid [VIA] or testing for HPV DNA. Each of these methods has specific advantages, disadvantages and health systems requirements that countries should consider when planning screening programmes. Vaccinating girls and women before sexual debut, and therefore before exposure to HPV infection, provides an excellent opportunity to decrease the incidence of cervical cancer over time. Increasing awareness of women about risk of cervical cancer and of benefits of screening programme is crucial in prevention of the disease.

2.1. Screening: definition and principles

Screening is defined as a procedure used to identify specified diseases or particular condition among asymptomatic individuals. In contrast, diagnostics is defined as application of variety of tests to symptomatic individuals who actively seek health-care services to identify the cause of their symptoms.[40] The distinction between screening and a diagnostic test is whether the test is offered without individual consideration.[41] Screening tests are applied to large populations, therefore they should be relatively inexpensive, convenient, painless and safe.[42] For this reason they often have higher margins of error and are less accurate than diagnostic tests.

The sensitivity of screening test is a measure of how good the test is at identifying individuals with a given disease. Sensitivity is defined as proportion of persons with a given disease who are screened as positive.[43] The screening result is true positive when the screening result is positive and the person has the disease. The screening result is false positive when the screening result is positive but the person does not have the disease.

Figure (2) demonstrates the relationship between screening tests parameters and screened disease and formulae for calculating parameters of screening tests.

The diagnostic tests should have very high sensitivity to detect the disease, whereas sensitivity and specificity are balance between each other to detect people with and without likelihood of disease and affordable costs.

Fig 2: Parameters of Screening Test

		Disease present	Disease absent
Screening test	*Positive*	*True positive (A)*	*False positive (B)*
	Negative	*False negative (C)*	*True negative (D)*

Sensitivity = proportion of patients with disease in whom the finding is positive =A/A+C

Specificity = proportion of those without the disease in whom the test is negative =D/D+C

Positive predictive value = probability of disease in subjects with a positive test result= A/A+B

Negative predictive value=probability of absence of the disease in subjects with a negative test result= A/A+B

The specificity of the screening test is a measure of how good the test is at identifying unaffected subjects. Specificity is defined as the proportion of individuals without the disease who get a negative screening test result.[44] The screening result is true negative when the screening test is negative and the person does not have the disease and, the screening test is false negative when the screening test is negative but the person has the condition.

Sensitivity and specificity are inversely related to each other. When sensitivity is increased, more persons with the disease are detected but also more persons who do not have the disease receive a positive screening result which is considered as false positive.

Positive and negative predictive values depend not only on the sensitivity and specificity of screening test but also on the prevalence of screened condition. The positive predictive value is probability of the disease in the subjects with positive result. The positive predictive value is higher for common disease than for rarer diseases. The significance of a positive predictive value depends very much on the consequence of a positive test. If it simply is followed by repetition of the screening test, the low positive predictive value might be well acceptable. If it is followed by a potentially harmful diagnostic examination it is important to achieve a high predictive value.[43] To be considered effective, a screening test must satisfy the requirements of efficacy and effectiveness of early detection. Efficacy of screening means that the test must be able to detect the target condition earlier that it would be without screening and with sufficient accuracy to avoid producing large number of false – positive and false –negative results. Effectiveness of early detection means that persons with disease who are detected early should have a better clinical outcome than those who are detected without screening.[45] In offering screening service for large number of symptom-free persons, and before starting the screening programme, very firm evidence is required to confirm that early diagnosis and any subsequent treatment will be better without harm. The possible harms can be in form of false positive screening test result, a wrong diagnosis, treatment which may do more harm than good, labeling people and false-negative findings that give false assurance. [46] False positive findings cause anxiety for healthy people, result in exposure of screened people to further examination, which may have risks. [42,44] Screening test has some psychological and social harms on screened women; these involve

anticipated discomfort or perception of adverse effects of screening test; unpleasant interactions with health care workers, anxiety over the results of a screening test implications of a positive screening test, and consequences of being labeled as sick or at risk of cervical cancer.[45] The result of screening test has different impacts on the screened people. People with a true positive finding and for whom the death is postponed, largely benefit from screening, but the benefit for those who have true positive finding but for whom death is not postponed, the value of screening is disputable. A false positive result causes moderate adverse effect, while false negative result causes minute undesirable effect and the true negative result has questionable value.[42] The objective of screening programmes is to reduce morbidity and mortality, and to improve the quality of life in the population.[41]

The criteria of screening programme, conditions to be screened and screening test were thoroughly described by Andermann et al [47] as follows:

A. The Wilson- Jungner criteria for appraising the validity of a screening programme

- The condition being screened for should be an important health problem.
- The natural history of the condition should be well understood.
- There should be a detectable early stage.
- Treatment at an early stage should be of more benefit than at a later stage.
- A suitable test should be devised for the early stage.
- The test should be acceptable.
- Intervals for repeating the test should be determined.
- Adequate health service provision should be made for the extra clinical workload resulting from screening.

- The risks, both physical and psychological, should be less than the benefits.
- The costs should be balanced against the benefits.

B. The criteria of screened condition

- The condition should be an important health problem.
- The epidemiology and natural history of the condition, including development from latent to declared disease, should be adequately understood and there should be a detectable risk factor, disease marker, latent period or early symptomatic stage.
- All the cost-effective primary prevention interventions should have been implemented as far as practicable.
- If the carriers of a mutation are identified as a result of screening the natural history of people with this status should be understood, including the psychological implications.

C. Criteria of screening test

- There should be a simple, safe, precise and validated screening test.
- The distribution of test values in the target population should be known and a suitable cut-off level defined and agreed.
- The test should be acceptable to the population.
- There should be an agreed policy on the further diagnostic investigation of individuals with a positive test result and on the choices available to those individuals.

If the test is for mutations the criteria used to select the subset of mutations to be covered by screening, if all possible mutations are not being tested for, should be clearly set out.

D. The treatment for screened condition

- There should be an effective treatment or intervention for patients identified through early detection, with evidence of early treatment leading to better outcomes than late treatment.
- There should be agreed evidence-based policies covering which individuals should be offered treatment and the appropriate treatment to be offered.
- Clinical management of the condition and patient outcomes should be optimized in all healthcare providers prior to participation in a screening programme.

2.2. Criteria of screening programme

UK National Screening Committee published the following criteria for screening programme.[48]

- There should be evidence from high-quality randomized controlled trials that the screening programme is effective in reducing mortality or morbidity. Where screening is aimed solely at providing information to allow the person being screened to make an informed choice, there must be evidence from high-quality trials that the test accurately measures risk. The information that is provided about the test and its outcome must be of value and readily understood by the individual being screened.
- There should be evidence that the complete screening programme is clinically, socially and ethically acceptable to health professionals and the public.
- The benefit from the screening programme should outweigh the physical and psychological harm (caused by the test, diagnostic procedures and treatment).

- The opportunity cost of the screening programme (including testing, diagnosis and treatment, administration, training and quality assurance) should be economically balanced in relation to expenditure on medical care as a whole (i.e. value for money).
- There should be a plan for managing and monitoring the screening programme and an agreed set of quality assurance standards.
- Adequate staffing and facilities for testing, diagnosis, treatment, and programme management should be available prior to the commencement of the screening programme.
- All other options for managing the condition should have been considered (for example, improving treatment and providing other services), to ensure that no more cost-effective intervention could be introduced or current interventions increased within the resources available.
- Evidence-based information, explaining the consequences of testing, investigation, and treatment, should be made available to potential participants to assist them in making an informed choice.
- Public pressure for widening the eligibility criteria for reducing the screening interval, and for increasing the sensitivity of the testing process, should be anticipated. Decisions about these parameters should be scientifically justifiable to the public.
- If screening is for a mutation, the programme should be acceptable to people identified as carriers and to other family members.

2.3. Impact of screening programmes on morbidity and mortality of cervical cancer

Screening programmes for cervical cancer were introduced in the 1950s and 1960s in many developed countries, especially in Nordic countries. Pap smear test has been used in these programmes to identify the presence of precursor lesions by using cytological investigation. Effectiveness of established screening programmes has been evaluated by observational studies and incidence and mortality rates of cervical cancer from cancer registries and mortality registers.[49] Cervical cancer incidence and mortality rates have significantly decreased in these countries due to implementation of screening programme. Time trends in mortality from cervical cancer in Denmark, Finland, Iceland, Norway, and Sweden since the early 1950s were investigated in relation to the extent and intensity of organised screening programmes in these countries. In all five countries the cumulative mortality rates (0-74 years) fell between 1965 and 1982. In Iceland, where the nationwide programme has the widest target age range, the fall in mortality was greatest (80%). Finland and Sweden have nationwide programmes also; the mortality fell by 50% and 34%, respectively. In Denmark, where about 40% of the population is covered by organised programmes, the overall mortality fell by 25%, but in Norway, with only 5% of the population covered by organised screening, the mortality fell by only 10%. The results support the conclusion that organised screening programmes have had a major impact on the reduction in mortality from cervical cancer in the Nordic countries.[50] In different counties of Denmark, incidence of cervical cancer observed high decrease in women aged 30-59 in relation to intensity of screening programme.[51] In Sweden cervical cancer mortality trends in relation to age, calendar period, county and degree of screening activities in the population were analyzed and 53% reduction in cervical cancer was found and it was attributable to screening.[52] Cohort studies have been done to estimate the risk of cervical cancer in screened and unscreened women. In British Columbia, Canada, age-adjusted relative risk for cancer in never-screened versus ever screened population was 6.[53] In

Finland, the risk of developing an invasive cancer in women aged 30-59 during the national screening programme in 1963-1971 was 0.2 in screened women, compared with average incidence in all women in period before screening. In non-participated women, the risk was1.6; thus the protective effect of screening programme was 58%.[54] In Sweden, linking of population register and screening register in two counties was used to calculate incidence rate between 1968 and 1992 in relation to screening history. Overall, relative risk in ever-screened versus never-screened was 0.55, but it was lower (0.27-0.38) in the age group 40-59 years.[55] The duration of the protective effect of Pap smear screening test was shorter in women below the age of 40 years than in older women; the protective effect of the Pap test seems to be stronger for shorter intervals [56]

The burden of disease from cervical cancer is under-appreciated in many countries, and there is a poor understanding of principles of effective prevention. A key barrier to implementation of effective cervical cancer prevention activities is lack of awareness and absence of political will to address the problem.[3] Research and experience has revealed that cervical cancer could be prevented when strategies and services are well-planned and well-managed and when attention is paid to programme monitoring and evaluation.[57] Many developed countries that have implemented well-organized screening and treatment programmes over the last 40 years have experienced dramatically reduced rates of cervical cancer.[58] However, in most low-resource countries where there are limited or no screening and treatment services, cervical cancer remains a leading cause of death among older women.[59]

The organization of cervical cancer prevention services depends on a number of factors, including status of current services; availability of resources; choice of screening test and treatment approaches; target ages; and screening interval selected. The consensus of the World

Health Organization (WHO) is that regions with limited resources should focus on screening women between ages of 30 and 49 years at least once in lifetime, gradually expanding the programme to other age groups and then more frequent screening and ensuring that women with positive results of testing for precancerous lesion are successfully treated.[60] Screening programme should achieve high coverage of the population at risk, to screen women with an accurate test as a part of high quality services and to ensure that women with positive result test are properly managed, and attracting and recruiting a large number of women in correct age groups.[61]

Follow-up of screened women can be performed in different time intervals. Screening every three or five years has almost as great impact as screening every year. Pap smear screening every three to five years with appropriate follow-up can reduce cervical cancer incidence by up to 80%.[62] Mathematical models on the impact of different types of screening programmes in South Africa suggest that if it were possible to provide a two-visit programme, in which all women receive an once-in-a-life Pap smear and follow-up with colposcopy and treatment, 19% reduction in cervical cancer would result.[63] Single-visit approaches using HPV DNA testing or VIA screening methods were found more effective and less expensive, once-in-a-lifetime screens using such approaches would reduce life-time cervical cancer risk by between 26% and 30%, compared with no screening. In Thailand, it was estimated that screening every five years would reduce cervical cancer incidence by 11% if cytology were used, by 20% if HPV testing was used and by 31% if VIA screening method was used.[64] In India, use of single round of VIA screening method resulted in a significant 25% reduction in cervical cancer incidence and a significant 35% reduction in cervical cancer mortality in the intervention group compared to the control group. This finding indicates that VIA is a

simple and effective method to prevent cervical cancer and death among deprived populations in developing and developed countries.[65]

2.4. Cervical cancer screening methods

Cervical cancer screening is a way of preventing cervical cancer from developing, and diagnosing the disease at an early pre-cancerous stage. Different methods are commonly used to which include:

2.4.1. Cytology screening test

Although the efficacy of cytology screening has never been established by randomized trials, it is commonly agreed that it has been effective in reducing the incidence of and mortality from cervical cancer in developed countries.[66,67] Well-organized programmes have shown the greatest effect, while using fewer resources than the unorganized programmes.[68] However, in all countries that think about introducing screening, this should be set within the context of planning nation-wide programme, and with full attention to programmatic issues.[69] Data from the International Agency on Research for Cancer (IARC) on cancer mortality proved major reductions in cervical cancer mortality in the Nordic countries that implemented organized programmes in the 1960s, and in United States of America and Canada where major efforts were made to encourage screening in the 1960s, though as yet organized programmes are not in place in North America. In the United Kingdom a major effort was started in 1988 to initiate organized programmes, and a substantial reduction in cervix cancer mortality is observed.[70] On the contrary, in the majority of developing countries screening reveals to have had slight or no effect with the exception of the programme in Chile.[71]

2.4.1.1. Validity of cytology screening test

There is common consensus that cytology is a highly specific screening test, and it specificity is estimated to be in the range of 95–99%.[69] Meta-analysis was used to estimate accuracy of Pap smear test . Several of these studies evaluated cytology cross-sectionally as a diagnostic test, rather than as a screening test. The cross-sectional studies of this meta-analysis included several studies suffering from verification bias. A few studies have assessed sensitivity of cytology longitudinally, using cancer as the endpoint. All were conducted several years ago in developed countries with high quality laboratories and they produced estimates of sensitivity ranging from 60% to 90%.[72,73]

Poor sensitivity in the laboratory will be compounded if adequate smears are not taken, as there are two components of false negatives, those that were caused by poor smear-taking, and those that were caused by laboratory error.[74] Cytology also suffers from relatively low reproducibility.[75,76] To reduce the impact of these deficiencies, there are many essential elements for successful cytology-based screening programmes.

2.4.1.2. Prioritization of age group to be screened

The priority age group to be screened should be defined by the age-related incidence of invasive cancer of the cervix in the country, not on the basis of the percentage distribution by age of clinically detected cases of cancer in the country. In most countries, it will be found that the majority of smears are being performed on young women, who are at low risk of presenting with invasive cancer within the next five years. Almost invariably it will be determined that the priority age group for initial screening is 35–54 years.[69]

2.4.1.3. Adequacy, fixation and preparation of Pap smear

Major causes of false negative results are insufficient collection of smear material from the transformation zone and inadequate preparation, fixation and processing of the smear.[75, 76] Use of spatula, combination of the spatula and endocervical brush allow adequate collection

of the target zone for preparation of conventional smears. The use of spatula or cotton tip applicator alone should be avoided.[77, 78] The speed of fixation is very important (the time between spread of material on the glass and fixation should be minimized to a few seconds). Fixation with alcohol has been shown in field circumstances to be adequate. Commercial fixative sprays are an alternative, but are more expensive. Smear-takers also need sufficient training. Several illustrated guidelines are available and are very useful tools.[79,80]

The laboratory should introduce a mechanism to monitor the proportion of inadequate smears submitted by the individual smear-takers. Those with >10% inadequate smears should undergo hands-on retraining in smear taking.[69]

2.4.1.4. Efficiency and quality of laboratory services

High quality laboratory services are essential to effective cytology screening. If it is possible to solve transport problems, the greater the centralization of such services the more efficient the laboratory will be. In small countries, this could imply a single central laboratory. In large countries, several regional laboratories will be required. In any case, a minimum throughput will be required to ensure adequate quality and efficiency. This minimum has been variously defined as 15–25,000 smears per annum [81,82] , or a work load justifying the employment of at least three technologists, who can each be expected to examine approximately 50 smears in an 8-hour day. The average time required for the interpretation of a one-slide gynecological smear by an experienced cytotechnologist is estimated to be six minutes.[83]

2.4.1.5. Quality control of cytology reading

Quality control programmes must be introduced in all cytology laboratories. A 10% full re-screening of negative smears is ineffective and is not recommended. Rapid re-reading of

100% of negative slides is effective, but may be outside the reach of low resourced programmes. Careful evaluation of detection rates by the smear reader and special evaluation of those with rates out of line with expectation may help to identify poor performers.[84]

2.4.1.6. Method to follow-up treated women

Follow-up of screened positive women should be in short period of time with smear, and with colposcopy to ensure whether the disease existed or not. Those with high-grade abnormalities should be followed annually for at least five years before they are returned to routine screening.[69]

The previous WHO recommendation was that when 80% of women aged 35–40 years have been screened once, screening frequency should increase to 10-yearly and then 5-yearly for women aged 30–60 years, as resources permit.[81] To date, data are not available to suggest that these recommendations should be revised. However, on the basis of modeling different approaches, it has been suggested that other intervals may be appropriate such as 5-yearly screening from the age of 35 for a total of three tests in a lifetime.[63] However, increasing the frequency of screening, and extending screening to younger age groups, does not compensate for deficiencies in laboratory quality or of population coverage.[69]

There has been some criticism for estimate methods used by IARC study group[85], to justify relatively infrequent screening frequencies. The assumption of frequency of screening is proven by the success of the program in Finland which was based on 5-yearly cytology screening for those age 35–59 years.[86]

Whatever the decision on the frequency of repeating screening, it will be necessary to actively invite women to return for screening when their next smear is due. The appropriate

mechanism will usually involve a similar mechanism to that used to invite women for their first smear.[69]

2.4.1.7. Strengths and limitations of cytology test

Cervical cytology is known to reduce cervical cancer incidence and mortality, particularly in organised programmes, though in North America and some countries in Europe benefit was obtained with excessive opportunistic screening. [69] In addition, the cytology test has the following strengths:

- Decades of experience in its use.
- High specificity.
- Lesions identified are easy to treat.
- Relatively low cost.
- Qualified manpower and laboratory resources exist in most countries.

However, there are limitations of the test. These include:

- The test is embarrassing and is difficult to comprehend in many cultures.
- Requires trained personnel.
- Smear adequacy is not intrinsically obvious. It is necessary to recall women for further tests if the smear is inadequate or for evaluation if an abnormality is suspected.
- In most laboratories only moderate sensitivity is achieved and reproducibility is poor.
- Cytology is unable to distinguish progressive disease from that destined to regress. This is true for both reported low-grade and high-grade lesions, with the probability of progression being much lower for low-grade abnormalities.

2.4.1.8. Consensus on cytology screening test

There is general consensus that cytology screening for cervical cancer has been effective in reducing the incidence and mortality from the disease in many developed countries. It is the well-organized programmes that have revealed the utmost effect, while using fewer resources than the un-organised programmes.

There is broad agreement that high quality cytology is a highly specific screening test, with estimates range of 98-99%. There is less agreement on the sensitivity of the test; cross-sectional studies have suggested sensitivity in the order of 50% in some circumstances. However, studies that have been able to assess sensitivity longitudinally have produced estimates that approximate to 75%.

The essential elements for successful cytology screening include: [69]

- Training of the relevant health care professionals, including smear takers, smears readers and programme managers.
- An agreed decision on the priority age group to be screened initially 35–54.
- Adequately taken and fixed smears; efficient and high quality laboratory services, that should preferably be centralized.
- Quality control of cytology reading; a means to rapidly transport smears to the laboratory.
- A mechanism to inform the women screened of the results of the test in an understandable form.
- A mechanism to ensure that women with an abnormal test result attend for management and treatment.
- An accepted definition of an abnormality to be treated, i.e. high grade lesions.
- A mechanism to follow-up treated women.

- A decision on the frequency of subsequent screens.
- A mechanism to invite women with negative smears for subsequent smears.

Elements that interfere with the development of successful cytology screening programmes include over-reliance upon maternal and child health services for screening, as women in their target group are generally too young, opportunistic rather than organized screening, and low coverage of the target group. Setting too low a threshold for referral for colposcopy, i.e. over-treating non-progressive disease, will lead to reduced cost-effectiveness.[69]

The major advantages of cytology screening are the considerable experience accumulated worldwide in its use, and that it is so far the only established screening test for cervical cancer precursors that has been shown to reduce the incidence and mortality of the disease. However, cytology has limitations; it is incompatible with some women's beliefs, and it is impossible to abolish the disease with screening. It is important that women are not coerced into screening, nor given an overoptimistic view of its potential. New developments in cytology, such as liquid-based cytology and automated reading have advantages, but are currently out of reach of most programmes.

2.5. Visual inspection screening methods

Even though cytology screening may be feasible in middle-income countries, there are technical, human resource and financial constraints in implementing such programmes in low-income countries. In view of this, alternative methods based on visual examination of the cervix have been investigated for the control of cervical cancer in low-resource settings.[87-89] The visual methods of screening include unaided visual inspection of the cervix visual inspection with 3-5% acetic acid (VIA) (synonyms: direct visual inspection (DVI), cervico-scopy, aided visual inspection, VIA with low-level magnification (VIAM),

cervicography, and visual inspection with Lugol's iodine (VILI). Down-staging has been shown to be inaccurate in detecting disease, particularly cervical pre-cancers [90], and is not further considered in this report. Among the visual inspection approaches, VIA has been more widely investigated for its performance characteristics (accuracy) in detecting cervical neoplasia, in various settings, and by different providers. VIA involves naked eye examination of the 3-5% acetic acid-swabbed uterine cervix without any magnification, usually by nurses and other paramedical health workers, with illumination provided by a bright light source, such as a halogen lamp. A positive test is the detection of well-defined, dull aceto-white lesions on the cervix. The objective of VIA is to detect aceto-white lesions leading to the early diagnosis of high-grade cervical intraepithelial neoplasia and early preclinical, asymptomatic invasive cancer. A major advantage with VIA is that it is a real-time screening test, as the outcome is known immediately after the administration of the test, so that further investigations/treatment can be planned and carried out during the same visit. Historically, before the advent of Pap smears and routine cytology-based screening programmes, health care providers relied on inspection of the cervix to detect abnormalities. After the 1950s, when cytology smears became the standard for cervical screening, the colposcopy initially developed in the 1930s began to be used increasingly to further investigate screen-positive women and to direct biopsies in order to confirm screening findings. Eventually, VIA was explored as an adjunct to the Pap smear to decrease the false negative rate of cytology and for more efficient identification of women for colposcopic triage. These studies, and the need for a suitable alternative for cervical cytology, led to the investigation of the accuracy and efficacy of VIA as a primary cervical screening tool. Moreover, they have provided valuable insights into the test characteristics of VIA in detecting cervical neoplasia. The results indicate that VIA is at least as sensitive

as conventional cytology in detecting high-grade lesions, but that its specificity is lower. Thus, VIA appears to be the most promising low-technology alternative to cytology.[69] VIA is currently being investigated for its efficacy in reducing incidence of and mortality from cervical cancer.

2.5.1. Current evidence on VIA test

The basic step in assessing the utility of a screening test is the determination of its test characteristics in terms of sensitivity, specificity and predictive values. Consistently low sensitivity and specificity of a given test preclude its further evaluation for reducing incidence and/or mortality from a given disease. Ottaviano and La Torre [91] examined 2400 women using VIA and the colposcopy. VIA detected abnormalities in 98.4% of patients assessed colposcopically as having an abnormal transformation zone and it correctly identified 98.9% of normal cases. In a study involving 145 women attending health clinics, the reported odds ratio for a positive cytology was 6.6 if the VIA test was also positive.[92] In a study among 2827 women, Slawson et al [93] demonstrated that VIA might be helpful in reducing referrals for colposcopy. Van Le et al [94] found that VIA resulted in an additional 15% of CIN cases being identified among cytology-negative women, but 40% of women with positive VIA underwent unnecessary colposcopy (false positives). Frisch et al [95]found that combining a negative cytology and negative VIA test resulted in a negative predictive value (NPV) of 91%greater than that obtained for cytology alone, but with some loss in positive predictive value (PPV). These studies demonstrated the potential value of VIA as a viable screening approach, but did not establish its test qualities as a primary screening method. Cecchini et al [96] provided evidence on the accuracy of VIA. VIA was more sensitive than cytology, but less specific. Additionally, screening sequentially using VIA was more cost− effective than with cervicography. Subsequently, six published studies on

VIA as a primary screening modality has been carried out in developing countries. In the study by Megevand et al [97] in South Africa, VIA and cytology were performed in a mobile unit equipped to process smears on site. In that setting, VIA detected 65% of high-grade squamous intraepithelial lesions (HSIL) confirmed by the reference standard. In another study from South Africa, Denny et al [98] compared performance of VIA, cytology and three other tests including HPV testing, all performed in a primary health care clinic. VIA and HPV testing were similar to cytology in their ability to detect HSIL+. Three Indian studies in the late 1990s provided additional evidence on the performance of VIA as an alternative to cytology as a primary screening test. Londhe et al [99] studied 372 women who underwent VIA, cytology and colposcopy in a gynecology outpatients clinic. VIA identified 78% of HSIL diagnosed through colposcopy 3.5 times more than those identified via cytology. Sankaranarayanan et al. [100] studied 3000 women, who had VIA and cytology provided by trained cytology technicians. The performance of both tests had sensitivity ratio of 1.05 in detecting moderate and severe dysplasia. In another study conducted by Sankaranarayanan et al in 1999 in India, nurses were trained to provide VIA and conventional cytology. About 1351 women were recruited and all recruits were subjected to both VIA and conventional cytology. VIA detected more ($P < 0.001$) LSIL and HSIL lesion than cytology but VIA was only 68% specific as compared to 90% of cytology.[101] In these studies, the reference investigation by colposcopy was carried out only in test-positive women and a small proportion of test-negative women, with the result that these studies suffered from verification bias.[76] It is quite likely that sensitivity may have been over-estimated as a result of verification bias, although the extent of this bias is difficult to assess because it is a function of the true prevalence of disease in each setting.[102] A study from Zimbabwe comparing VIA and cytology performed by nurses in primary health clinics was the first to

yield direct estimates of sensitivity/ specificity, because all women testing negative or positive on screening were offered the reference standard, thus avoiding verification bias.[103] In that study, the sensitivity of VIA (for HSIL +) was 1.75 times higher than cytology (76.7% versus 44.3%, respectively), whereas the specificity was 1.4 times lower (64.1% versus 90.6%). The range of estimated VIA sensitivity from the seven cross-sectional studies that specifically addressed the accuracy of VIA was 66% to 96% (median 84%) [95-101]. For specificity, the range was 64% to 98% (median 82%). The positive predictive value ranged 10-20% and the negative predictive value 92-97%. The weighted mean sensitivity and specificity of VIA from these studies were 81% and 83%, respectively. Of interest, the above ranges are considerably narrower than those observed from cross-sectional cytology studies (i.e., 20-85%) over the past few decades.[72,73] In studies where VIA was compared to cytology in the same setting, VIA performed similarly to cytology in terms of detecting high-grade lesions or cancer, but was less specific. [96-100] The addition of magnification to VIA(VIAM) does not seem to improve the accuracy of the test.[98] A useful complement to this qualitative review of the evidence on VIA to date would be a meta-analysis aimed at providing a quantitative summary measure of test performance indicators of VIA. One such analysis involving three VIA studies conducted before 1996 compared the ability of VIA, cytology and a number of other tests to identify any precancerous lesions.[104] VIA had a substantially higher area under the receiver operating curve (0.85) compared to cytology (0.70) in this study. Given that VIA test performance indicators (especially sensitivity) from more recent studies are generally higher than those from the three studies used in that analysis, future meta-analyses will likely provide even more convincing evidence on the test characteristics of VIA. Thus,

studies to date support the conclusion that VIA performs similarly, if not better than cytology in the detection of high-grade cervical cancer precursors.[69]

2.5.2. Advantages and limitations of VIA screening test

The advantages and limitations of VIA as a screening test are presented in Table 4. Many aspects of VIA make it an attractive test for use in low-resource settings. It is a simple, inexpensive, low-technology test that requires minimal infrastructure for use. Its cross-sectional sensitivity appears to be similar to cytology in detecting high-grade disease. It is possible to train workers on how to use this screening method in a short period of time (1−2 weeks). It is a real-time test in the sense that the results are available immediately, making it possible to institute further diagnostic investigations for test positive women, as well as plan and offer treatment during the same visit. The test appears to be comparable in reproducibility to other tests. VIA based screening programme may be readily integrated in the primary care level of health services. However, the low specificity of VIA may result in over-investigation and possible over-treatment in test and treat conditions. The test positivity rate varies from 10–35% in most reported and ongoing studies. Adequate training of health workers is important to reduce false-positive referrals. To date, no standard quality control procedures are available for VIA. The test essentially identifies disease in the ectocervix only when the transformation zone remains on the visually exposed part of the cervix. Since the transformation zone recedes to the endocervical canal in postmenopausal women and the test has inherent difficulties in identifying endocervical disease, VIA may be of limited use in older women. How well VIA works in an integrated service delivery model, with other competing demands for provider time, knowledge and skills, is not yet proven – most of the data are from research settings in which dedicated

providers largely perform VIA only. Performance decay over time may be an important problem to tackle.[69]

Table 4: advantages and limitations of VIA test

Advantages	Limitations
• Simple and easy to be done; • Low cost; • Can be done in low – resource settings; • Consistent across all studies in different designs; • High sensitivity in detecting high grade lesion; • Easy of training screeners with different medical background within days; • Single visit screening and result; • Safe and effective treatment.	• Low specificity; • High positive rate; • Low positive predictive value; • No standardized method of quality control.

2.6. Cancer situation in Sudan

Sudan is geographically situated in northern African region and belongs to Eastern Mediterranean Regional Office (EMRO) based on WHO member states. Cancer situation in Sudan has same determinants of cancer as in other African countries. Risk of getting cancer before the age of 75 year was 8.6% for the whole population; estimated number of new cancer cases per year was 21900 estimated number of cancer deaths was 16700 per year; and age-standardized rate was 81.6 per100, 000. The five most frequent cancers, were cancers of breast, non-Hodgkin lymphoma, leukemia, esophagus, and colorectum; among women cancers of breast, cervix, ovary, esophagus and non-Hodgkin lymphoma; among male on the other hand, cancers of non-Hodgkin lymphoma, prostate, leukemia, colorectum and liver.[2] Current burden of invasive cervical cancer in Sudan with estimates of annual indicators and number of new cases is shown in Table 5. Incidence of cervical

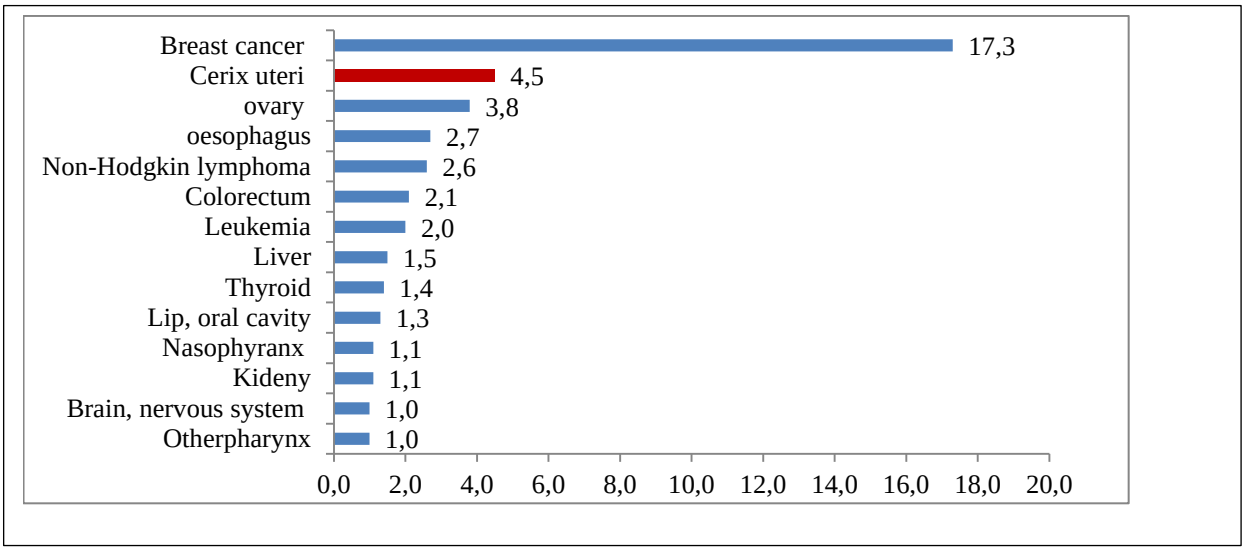
cancer was estimated to be the second after breast cancer among women in Sudan (Fig. 5). Estimated crude mortality rate of cervical cancer was 3.0 per 100,000 (Fig. 6).[2]

Table 5 : Incidence of cervical cancer rates (per 100.000 women year) in Sudan, Northern Africa and the world [2]

Indicator	Sudan	Northern Africa	World
Crude incidence rate	4.5	5.2	15.8
Age standardized incidence rate	7.0	6.6	15.3
Cumulative Risk (%) age 0-70 year	0.7	0.8	1.6
Annual new number of cases	923	5278	529828

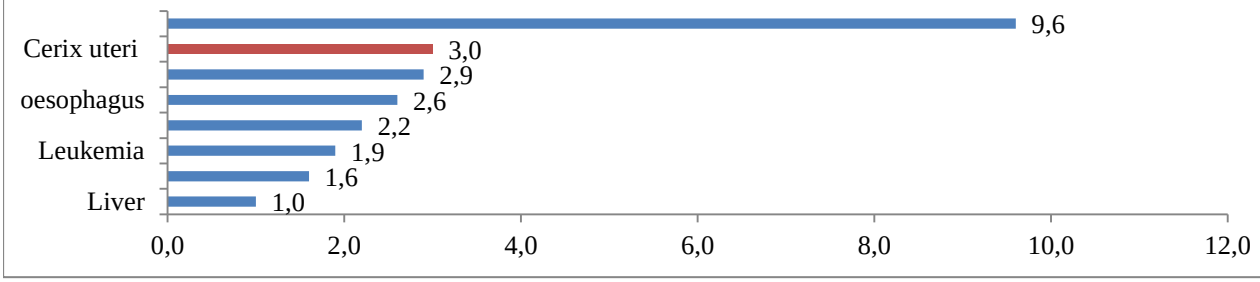
Standardized rates have estimated using direct method and world population as reference

Fig 5: Incidence of cervical cancer annual crude rate per 100,000 compared to other cancers among women in Sudan [2]



There is no population–based cancer registry in Sudan, but there are two small units of hospital-based cancer registry at Radiation and Isotope Center in Khartoum (RICK) and Cancer Institute at University of Gezira. However, the registration of cancer cases at these two units is limited to the cases which received health care at the two health care settings.

Fig 6 : Annual crude cancer mortality rate per 100000 among women in Sudan [2]



There is one specialized hospital for diagnosis and treatment of cancers in Sudan. However, there are no preventive cancer services; neither screening programmes nor education. There is no comprehensive research agenda on cancer due to scarcity of resources and competition from others health priorities

3. Aims of the study

In the absence of screening programme this study investigated different aspects of cervical cancer situation in Khartoum State: risk factors for VIA positivity; predictors of advanced stages at diagnosis; and performance of two screening tests of VIA and Pap smear. In addition, assessment of feasibility and acceptability of visual inspection with acetic acid among screened women was done. Moreover, knowledge and practice of physicians about control and preventive services of cervical cancer in the study setting were assessed.

3.1. General aim

To provide evidence-based epidemiological information about cervical cancer and to assess the feasibility of screening to help to initiate cervical cancer preventive services in Khartoum state, Sudan.

3.2. Specific aims

3.2.1. To study the risk factors of VIA positivity in Khartoum state, Sudan.

3.2.2. To determine feasibility and acceptability of visual inspection with use of acetic acid (VIA) as screening method for cervical cancer as an alternative to Pap smear in primary health care setting in Sudan.

3.2.3. To study predictors of diagnosis of cervical cancer at advanced stages in Sudan.

3.2.4. To compare sensitivity, specificity, positive and negative predictive values and histological diagnosis of positive cases of VIA and Pap smear tests.

3.2.5. To assess knowledge and practice of physicians about cervical cancer screening in Khartoum state, Sudan.

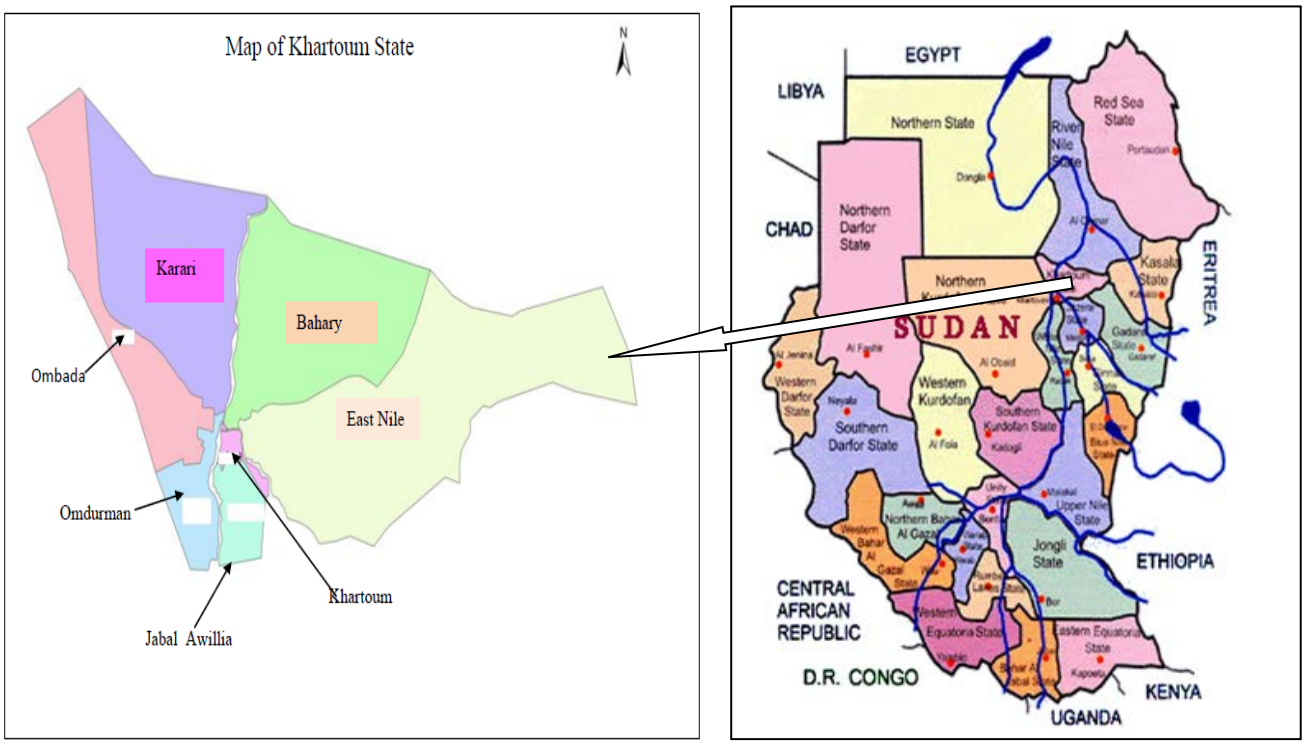
4. Research methods

4.1. Study area

Khartoum is the federal capital of Sudan and capital of Khartoum state. The Khartoum state situates in the northern part of Sudan in longitude lines 34.24° east, 31.53°west, and latitude lines 15.9°south and 16.45° north; with total land area of 22736 km^2. Total population of Khartoum state was 6409295 based on the census of 2008. About 86% of the populations live in urban area and 14% live in rural area.

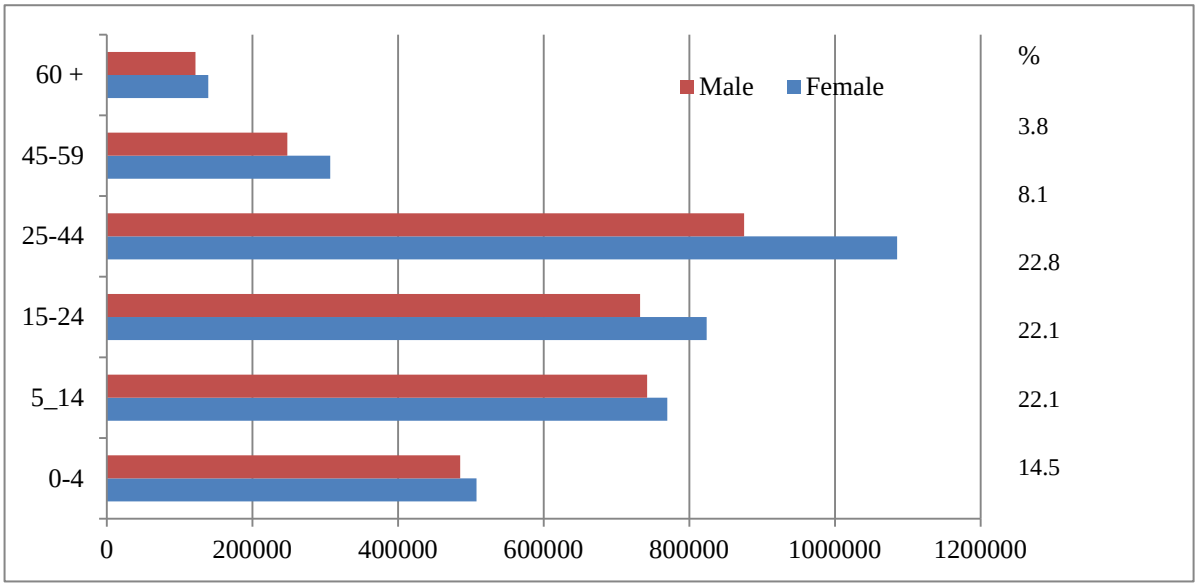
Khartoum state consists of seven localities, which are Bahary, East Nile, Khartoum, Karari, Jabal Awailia, Omdurman, and Ombada (Figure.7).

Figure 7: Map of Khartoum state



According to the annual report for the year 2008 by Ministry of Health of Khartoum state the estimated total population was 6409295 with population growth rate of 3.7, and male to female ratio 1.0:1.1.The population pyramid shape is typical to that of developing countries, in which the age group less than five years represents about 14.5% of the population and the age group 60+ consists only 3.9% of the total population (Figure 8). In Khartoum state the crude birth rate was 33.7 per 1000, crude death rate 8.8 per 1000;women in childbearing age consist about 44% of total women population and fertility rate is 4.7%.

Figure 8: Population in Khartoum State based on the census in the year 2010



There were 28 hospitals and 156 primary health care centers in Khartoum State. Doctor-to-population ratio was 16 per 100,000 and for nurse/midwife ratio was 4.7 per 100,000.

4.2. Study design

Descriptive, cross-sectional surveys were conducted in four consecutive phases during the study period. In the first phase a pilot study was undertaken to study risk factors of cervical cancer; in the second phase a hospital –based survey was carried out in which data were collected from the cancer registry unit to determine predictors of different stages of cervical cancer at diagnosis. In the third phase, a definitive study was carried out to determine the performance of VIA test compared to Pap smear, and in the fourth phase a survey was conducted to assess knowledge and practice of physicians about cervical cancer screening.

4.3.Study population

In the pilot study the study population was asymptomatic women living in Khartoum State in Sudan. In the second survey, the target population was women who were diagnosed with cervical cancer and registered at the cancer registry unit in Radiation and Isotopes Center in Khartoum (RICK) in the time period of January to December 2007. In the third survey, which

was the definitive study, the target population was healthy women aged 25-49 years who were living in Khartoum state. In the fourth survey the target population was physicians who were working in obstetrics and gynaecologic health care service in Khartoum State.

4.4. Study subjects recruitment methods

Before the pilot study was conducted, six nurses and two supervisors were trained on pre-screening counseling, data collection and VIA screening methods in a primary health care center setting by the investigator and gynecological oncology consultant from RICK. A campaign to raise public awareness about cervical cancer among the local women was performed. Posters and pamphlets were distributed to women in public places at primary health centers, shopping centers, and transport centers. A programme about cervical cancer and VIA screening methods was presented by a gynecological oncologist and investigator at the local radio broadcasting station. In addition, in the context of Friday and Sunday prayers, Imams in mosques and clerks in churches talked about cervical cancer screening and encouraged women to participate in the screening project. House-to-house visits were used to recruit participants. During the home visits, pamphlets were distributed, and women were informed about cervical cancer and about the opportunity for prevention and treatment by early detection. Healthy non-pregnant women aged 25–50 years living in Khartoum State in Sudan, who were voluntarily willing to participate in screening, were included in the study. Eligible women were given an appointment for screening at the cervical cancer screening clinic. This method was applied in the pilot study and in the definitive phase of the screening project.

4.5. Sample size and selection methods

Data about married women age 25-49 years were obtained from Statistic Department in Khartoum Ministry of Health in Khartoum state. The total number was 25200 women. Based on GLABCAN statistics for year 2008, the prevalence of cervical cancer in Sudan was 8.8%.[2]

Sample-size calculation

The appropriate sample size for a population-based survey for pilot study (Study No. 1) and definitive study (Study No. 3) is determined by three factors:

(i)Total number of target population

(ii)Proportion of target population from total population (P)

(iii)Non- response rate (NRR)

Description:

n= sample size

N= total target population = 25200

P = proportion of target population from total population =13.6

NRR= non- response rate accounts for women that could be either absent, not accessible, refuse to be surveyed, or any other reason that prevent survey teams from surveying a selected participant. NRR is used to correct sample size. 50% of women assumed to be non- respondent to survey. The following formula is used to calculate NRR:

NRR= 1-0.05 = 0.95

Sample size is calculated by using the following equation:

n= N/P x NRR

n=25200/13.6x 0.95 = 1950.

Sample size = 1950 women

Study No.4: the investigator targets all of physicians study area who practice obstetrics and gynecology (n= 230). Sample sizes for pilot study and definitive study were predetermined by investigators based on available resources.

4.5.1. The pilot study: Sample frame was prepared by listing all study target population size of 25200 married women age 25-49 years in study area. Required sample size was predetermined by investigator to be 168 women. Sampling ratio (*sampling fraction*) was calculated = target population units (N) /number of study units (n).[105] Participants were randomly selected from the list of 25200 women using systematic random sampling. Sample fraction was used for selection of required sample size (25200/168=150). Therefore from each 150 women one woman was randomly selected from the list. The total number of selected women was 168, of which 18 women were ineligible to participate and thus the remaining number was 150 women.

4.5.2. The definitive study: A sample size of 1950 women was predetermined. Systematic sampling method was used to obtain the required sample size. Sampling ratio (sampling fraction) was calculated = sample/number of study units = (25200/1950=13). Using the systematic sampling method, one woman in 13 women was selected from the list. This assured that every woman in the target population had an equal chance to be selected. House-to-house visits were performed to all selected women (n=1950). During the visits 1250 women were accessed and they received health education about cervical cancer and how it can be prevented through regular screening. Thereafter the 1250 women were invited to attend the cervical cancer screening programme at RICK, of whom 79% (985/1250) attended screening.

4.5.3. The hospital-based survey: Data from 197 women diagnosed with cancer of the cervix during the period of the 1st January 2007 to the 30thDecember 2007 were collected for the study. The data were checked for completeness. The target study variables were: age, marital status, tribe, residence area, state, health insurance status, and tumor stage at diagnosis. Tumors were classified based on Staging Classifications and Clinical Practice Guidelines for Gynecological Cancers by Federation International of Gynecology and Obstetrics (FIGO) [11] which operates with four different disease stages. Stage I: where the cancer is strictly confined to the cervix; stage II where the carcinoma extends beyond the cervix, but has not extended onto the pelvic wall and involves the two thirds of upper vagina; stage III where the carcinoma has extended onto the pelvic wall and involves the lower third of the vagina but has not extended not to adjacent organs; and stage IV where the carcinoma has extended beyond the true pelvis and spreads into adjacent and distant organs. Staging is based on a combination of anatomical, pathological, operative, and clinical assessments.

4.5.4. The survey of physicians' knowledge and practice: A list of obstetricians /gynecologist and general practitioners was obtained from a sample of size of 10 hospitals. The total number of target physicians was 230, of whom 152 (66.1%) were from governmental hospitals and 78 (33.9%) from private sector. All physicians were targeted were invited to participated in the survey. The response rate was 69.6 % (160/230).

4.6. Sample size

Different samples sizes were randomly selected from the target population based on sample size calculation of the four surveys. The obtained sample sizes were: (n=150) in the pilot

study, (n=197) in the hospital-based survey, (n=1250) in the definitive study and (n=230) in the survey of knowledge and practice among physicians.

4.7. Data collection

Data were collected from the target study populations by using different methods**:** semi-structured questionnaire inquiring demographic, reproductive factors and other risk factors. Visual inspection with use of acetic acid (VIA) and conventional Pap smear methods were used to screen the participating women, followed by colposcopy and biopsy for confirmation of the positive results. A self–administrated questionnaire was used to collect data from physicians about their knowledge and practices of cervical cancer screening. Completed data of the diagnosed women with cervical cancer in year 2007 were obtained from the cancer registry unit at Radiation and Isotopes Center in Khartoum and analyzed to determine predictors of different stages of cervical cancer at diagnosis.

4.8. Data collection methods

Different methods were used for data collection as follows:

4.8.1. Structured interviews: The women in relation to the home visits were interviewed about their socioeconomic characteristics, reproductive history, and contraceptive use, history of sexually transmitted infections, genital mutilation, partner circumcision, episiotomy, cervical trauma, tobacco use and use of wood smoke as cosmetic in lower part of the body and other information (Questionnaire, Appendix No. 11.3)

4.8.2. Self-administrated questionnaire: A letter was sent to all selected physicians **(**Appendix No.12.4**)** together with the questionnaire **(**Appendix No.11.5). The questionnaire was developed and distributed to all physicians; it contained variables on job title, type of job, health care setting type, and work experience, knowledge of cervical

cancer screening methods and practice of cervical cancer screening. The questionnaire was completed by physicians and returned to the investigator.

4.8.4. Cervical cancer screening: Two screening tests were used: (a) Pap smear, in which smears were obtained from each participating woman and fixed with ethanol for 30 minutes and sent to cytologist for investigation. (b) VIA test was performed by adding 5 ml of acetic acid (vinegar) to 95 ml of distal water in a sterile kidney dish to compose 5% acetic acid. A sterile bivalve speculum was inserted into the vagina. The vagina wall and cervix were inspected for the presence of tumors and other diseases. Any mucus or discharge at the cervix was cleaned with the use of sterile cotton. Then, the squamo-columnar junction was determined and acetic acid was applied. VIA positive was defined as well-marginated, raised, opaque, aceto-white lesion at the squamo-columnar junction.[21] VIA-positive women were referred to the oncology gynecologist at the Radiation and Isotopes Center for treatment. VIA-negative women were assured and asked to have the test repeated after five years. Women who had other diseases such as bacterial or fungal infection were also treated at the screening center. The treatment was offered free of charge. Cytology and histopathology was obtained from by laboratory by use of laboratory form (Appendix 11.6)

4.8.5. In-depth interviews: To get a detailed description of women's notion of cervical cancer and how they perceived the screening activities offered, in-depth interviews were performed with 100 women. Further, to get an understanding of feasibility and acceptability of VIA screening method for cervical cancer among screened women, a thematic interview guide was developed; the interviewer used the guide to focus the discussion, but not limit to asking only the developed questions. The individual interviews were discussed in detail with the interviewers during the data collection. This

approach was taken to incorporate the experiences gathered during the interviews, to develop and add more in-depth insight. The interviews were conducted in Arabic and transcribed into English.

4.9. Data management and statistical analyses

Data were stored and analyzed by STATA Version 9.2 Stata Corp, Texas USA**.** Descriptive statistics were used to analyze the demographic data and to compare the results of both screening tests. T-test was used to detect any significant difference between continuous variables. Chi squared test was used to detect any significant difference between categorical variables and positive results of VIA and Pap smear tests in the screened women in each group. Logistic regression was used to assess the predictors of advanced stages of cervical cancer at diagnosis. Sensitivity, specificity, positive and negative predictive values were compared for both screening tests. P value and 95% confidence interval are reported.

9.10. Ethical clearance

The project proposal got ethical clearance from the Department of Health Research and Committee on Human Research Ethics in the Khartoum state (Appendix11.8). All recruited participants were informed about the objectives of the research and importance of cervical cancer screening. Informed consent was obtained by using specific form (Appendix.11.2). Women who had negative test were assured and those who had positive screening test in either or both screening tests were treated and those needing further management were referred to Isotopes and Radiation Center in Khartoum. The treatment was offered to all participants free of charge. Participants' information was handled with high confidentiality.

5. Results

5.1. Risk factors of VIA positivity (*Pilot study*)

One hundred and fifty women aged 25–50 years were invited to participate in the cervical cancer screening project, and 100 women attended the screening clinic (response rate of 67%). Table 6 shows the characteristics of the subjects. The mean age was 35 years, 36% had no education, and 33% were employed. Sixty-four percent of the participating women had had genital mutilation. Sixty percent were parous, 80% of them had spontaneous vaginal delivery, 62% were episiotomized, 12% had laceration and 30% of the screened women used contraceptive methods.

Table 7 describes the association between risk factors and positivity of VIA. A highly statistically significant association was observed between VIA positive and uterine cervix laceration (OR 18.6, 95% CI: 3.2-107.9; p=0.001); assisted vaginal delivery was also strongly related to positive VIA (OR 14; 95% CI: 3.2-61.1; p=0.0004). Risk of VIA positivity was significantly higher among parous than among nulliparous women (OR 5.8; 95% CI: 1.2–27.0; p=0.02). Moreover, this study showed that there was a no association between a positive VIA result and female genital mutilation (OR 0.7; 95% CI: 0.1-3.8, p=0.7). Furthermore, the results showed a statistically significant association between episiotomy and a positive VIA results (OR 5.0; 95% CI: 1.2–25.1; p = 0.04). All these associations remained statistically significant after adjusting for other factors, including age, educational level, and employment, and potential confounding factors such as smoking, number of sexual partners, and use of contraceptive methods.

No statistically significant findings were found for male partner circumcision, use of contraception method, or use of cosmetic smoking in the lower part of the body. This study revealed a high proportion of accessibility to the screening method. About 98% of screened women were satisfied with their decision to be screened.

5.2. Feasibility and acceptability of VIA test (*Pilot study*)

This study revealed a high proportion of acceptability to the screening method. About 98% of screened women were satisfied with their decision to be screened. A total of 81.6% of the participants stated that the visit to the screening clinic took less than 45 minutes; 90.8% mentioned that counseling before screening provided enough information about screening VIA test; 88.8% declared that test experience was better than expected; and 93% recommended the VIA to be performed to other women. Treatment of the confirmed cases of cervical cancer was postponed due to a lack of resources for two patients 12.5% (Table 8).

5.3. Predictors of advanced stages cervical cancer at diagnosis *(Hospital-based study)*

The mean age at diagnosis of the patients was 54.5 years (range from 25 to 76 years). About 70% of women in the study sample were currently married and more than half of them were living in rural area (Table 9). Health insurance rate was 27%. About 72% of women in the sample were diagnosed with invasive cervical cancer that had spread beyond the cervix.

Women who were diagnosed at advanced stage of cervical cancer were older than those diagnosed at early stage (Table 10). More than half of the cases were diagnosed at stage IV. Proportion of women who were diagnosed at advanced stages of cervical cancer was higher than of women who were diagnosed in early stage (71.5% versus 28.4%). Early cervical cancer stage was frequently diagnosed (30.1% versus 27.4%) among who age women ≤54 years than women who age ≥ 54 years respectively. While advanced cervical cancer stage was frequently diagnosed among women who age ≥ 54 years compared to women who age women ≤54 years (72.6% versus 69.9%) respectively. Women of African ethnicity had higher proportion of being diagnosed with stage IV compared to Arabic women. Women living in

urban areas had higher chance of being diagnosed with earlier stages compared to those living in rural area.

Table 6: Characteristics of the participants in cervical cancer screening with use of VIA method, Khartoum state, Sudan, n=100

Characteristics	Total	VIA test	
	(n=100)	Negative (n=84)	Positive (n=16)
	N (%)	N (%)	N (%)
Age			
mean age	37.2	34.1	40.3
≤ 35 years	52 (52)	45 (54)	7 (44)
≥ 36 years	48 (48)	39 (46)	9 (56)
Education level			
Secondary school	36 (36)	32 (38.1)	4 (25)
Basic school	64 (64)	52 (61.9)	12 (75)
Employment			
Yes	33 (33)	29 (34.5)	4 (25)
No	67(67)	55(65.5)	12(75)
Smoking			
Yes	4(4)	2(2.4)	2(12.5)
No	96(96)	82(97.6)	14(87.5)
Female genital mutilation			
Yes	90(90)	76(90.5)	14(87.5)
No	10(10)	8(9.5)	2 (12.5)
Male partner circumcision			
Yes	98 (98)	83 (98.8)	15 (93.7)
No	2 (2)	1 (1.2)	1 (6.3)
Contraception			
Yes	30 (30)	27 (32.1)	3 (18.8)
No	70 (70)	57 (67.9)	13 (81.2)
Use of cosmetic smoking			
Yes	97 (97)	82 (81.5)	15 (93.7)
No	3 (3)	2 (2.5)	1 (6.3)
Parity			
Nulliparous	40 (40)	38 (45.2)	2 (12.5)
Parous	60 (60)	46 (54.8)	14 (87.5)
Vaginal delivery (*out of parous n=60*)			
Spontaneous	48(75)	42 (91.3)	6 (41.9)
Assisted	12(25)	4 (8.7)	8 (57.1)
Episiotomy (*out of parous n=60*)			
Yes	37 (61.7)	25 (28.2)	12 (9.8)
No	23 (38.3)	21 (17.8)	2 (6.2)
Uterine cervix laceration			
Yes	7 (7)	2 (2.4)	5 (31.3)
No	93 (93)	82 (97.6)	11 (68.7)

Tale 7: Association between risk factors and VIA screening test in Khartoum state, Sudan (n= 100)

Risk factor	N	VIA(-)	VIA(+)	Odd Ratio	95% CI	P value
Uterine cervix laceration						
Yes	7	2(28.3%)	5(71.4%)	18.6	3.2-107.9	0.001
No	93	82(88.2%)	11(11.8%)			
Assisted vaginal delivery						
Yes	12	4(33.3%)	8(66.7%)	14.0	3.2-61.1	0.0004
No	48	42(87.5%)	6(12.5%)			
Episiotomy						
Yes	37	25(67.5%)	12(33.5)	5.0	1.0-25.1	0.04
No	23	21(91.3%)	2(8.7%)			
Parity						
Yes	60	46(76.7%)	14(23.3%)	5.8	1.2-27.0	0.02
No	40	38(95%)	2(5%)			
Female genital mutilation						
Yes	90	76(84.4%)	14(15.6%)	0.7	0.1-3.8	0.7
No	10	8(80%)	2(20%)			
Male partner circumcision						
Yes	98	83(84.7%)	15(15.3%)	0.2	0.01-3.04	0.2
No	2	1(50%)	1(50%)			
Use of contraception method						
Yes	30	27(90%)	3(10%)	0.5	0.12-1.85	0.3
No	70	57(81.4%)	13(18.6%)			
Use of cosmetic smoking						
Yes	97	82(84.5%)	15(15.5%)	0.4	0.03-4.29	0.4
No	3	2(66.7%)	1(33.3%)			

Table 8: Acceptability and feasibility of VIA screening method for cervical cancer among women in Khartoum State Sudan (n=98)

Variable	Yes (%)	No (%)	P value
Satisfied with the decision to be screened	96 (97.7)	2 (2.3)	0.0001
Visit to the screening clinic took less than 45 minutes	85 (86.7)	13 (13.7)	0.0001
Counseling provided enough information about screening	89 (90.8)	9 (9.2)	0.0001
Waiting time before screening was less than 20 minutes	82 (83.7)	16 (16.3)	0.0001
Screening test procedure was less than 25 minutes	79 (80.6)	19 (19.4)	0.0001
Screening test experience was better than expected	87 (88.8)	12 (12.4)	0.0001
Was informed immediately about the result after the screening test	95 (96.9)	3 (3.1)	0.0001
Recommended screening test to other women	91 (92.9)	7 (7.1)	0.0001
Treatment was offered free of charge	12 (87.5)	2 (12.5)	0.0001

The relationship between predictor variables and the stage of cervical cancer at diagnosis was examined using multivariate logistic regression (Table 11). Age, ethnicity, residency and health insurance status were associated with advanced stages of diagnosis. Age was associated with increased odds of advanced stage diagnosis (OR, 1.03, 95% CI, 1.01-1.05). Being a rural area resident was associated with increased odds of advanced stage diagnosis

(OR, 1.13, 95% CI, 1.78-5.50). Advanced stage diagnosis was far more likely among women of African ethnicity than among women of Arab ethnicity (OR, 1.76, 95% CI; 1.01-3.05). Women who were not covered by a health insurance had greater odds of advanced stage at diagnosis of cervical cancer than women who were covered (OR, 8.6, 95% CI; 4.55-16.2)

Table 9: Characteristics of women diagnosed with cervical cancer(N=197) in the RICK, Sudan, in 2007

Characteristics	N (%)
Age (years)	
<54	73 (37.1%)
≥54	124 (62.9%)
Marital status	
Single	60 (30.5%)
Married	137 (69.5%)
Ethnicity	
African	103 (52.3%)
Arabic	94 (47.7%)
Education level	
Basic school	122 (61.9%)
Secondary School	75 (38.1%)
Geographical area	
Urban	90 (45.7%)
Rural	107 (54.3%)
Health insurance	
Covered	53 (26.9%)
Not covered	144 (73.1%)
Tumour morphology	
Squamous cell carcinoma	145 (78.2%)
Adenocarcinoma	52 (26.2%)
Disease stage at diagnosis (FIGO classification)	
Stage I	17 (8.7%)
Stage II	39 (19.8%)
Stage III	27 (13.7%)
Stage IV	114 (57.9)

Table10: Distribution of predictors and stages of cervical cancer at diagnosis (n=197).

Predictor	Early stage I&II (n= 56 (28.6%)	Advanced stage III&IV (n=141 (71.5%))	*P* value
Age group (years)			
<54	22 (11.1%)	51 (25.9%)	0.8
≥55	34 (17.3%)	90 (46.7%)	
Marital status			
Single	16 (8.1%)	44 (22.3%)	0.7
Married	40 (20.3%)	97 (49.2%)	
Educational level			
Primary school	34 (35.1%)	88 (44.7%)	0.8
Secondary school	22 (11.2%)	53 (26.9%)	
Ethnicity			
Arabic	38 (19.3%)	56 (28.4%)	0.003
African	18 (19.3%)	85 (43.1%)	
Geographical area			
Urban	38 (19.3%)	52 (26.4%)	0.001
Rural	18(19.3%)	89(45.1%)	
Health Insurance			
Insured	32 (16.2%)	21 (10.7%)	0.0001
Not insured	24 (12.2%)	120 (60.9%)	

Table 11: Multivariate logistic regression analysis of predictor variables of stage of cervical cancer at diagnosis*

Predictor	Early stages (I&II)	Advanced stages(II&IV)	Unadjusted OR 95% CI	Adjusted OR (95% CI)	*P value*
Age	34 (17.3%)	90 (46.7%)	1.1 (0.60 -2.15)	1.1 (0..60- 2.15)	0.7
Marital status	40 (20.3%)	97 (49.2%)	0.8 (0.44- 1.74)	0.8 (0.43-1.45)	0.7
Education level	34 (35.1%)	88 (44.7%)	0.9 (0.49 – 1.75)	0.7 (0.43-1.32)	0.9
Ethnicity	18 (19.3%)	85 (43.1%)	3.2(1.66 – 6.16)	1.76 (1.01-3.05)	0.003
Residency	18 (19.3%)	89 (45.1%)	3.7 (2.54 -9.31)	1.13 (1.78-5.50)	0.001
Health insurance coverage	24 (12.2%)	120 (60.9%)	8.6 (4.55-16.24)	7.7 (3.76 -15.38)	<.0001

*Reference category: older age ≥54 year, marital status married, education level: primary school, African ethnicity, residency in rural area, uncovered by health insurance. Adjusted for: younger age ≤54 years, married, education level secondary, Arabic ethnicity, residency in urban area, cover by health insurance

5.4. Characteristics of the participants in the VIA and Pap smear screening *(definitive study)*

Figure 9 demonstrates a flow chart of the study (appendix No.11.1). Total number of women who were invited to participate in the study was 1250 women; of them 79% (985/1250) agreed to participate in the study. Of the total number of responded women, 5.2% (51/985) were excluded due to different reasons: 2.5% (25/985) had absence of menstrual period, 1.6% (16/985) were not sure about their last menstrual period, 0.3% (3/985) had previous history of cervical cancer treatment and 0.6% (6/985) were under treatment for infertility problems. The percentage of women, who fulfilled the eligibility criteria and agreed to participate in the definitive study from the total number of respondents, was 95 % (934/985). Eligible participants received counseling on screening procedures and completed informed consent procedure.

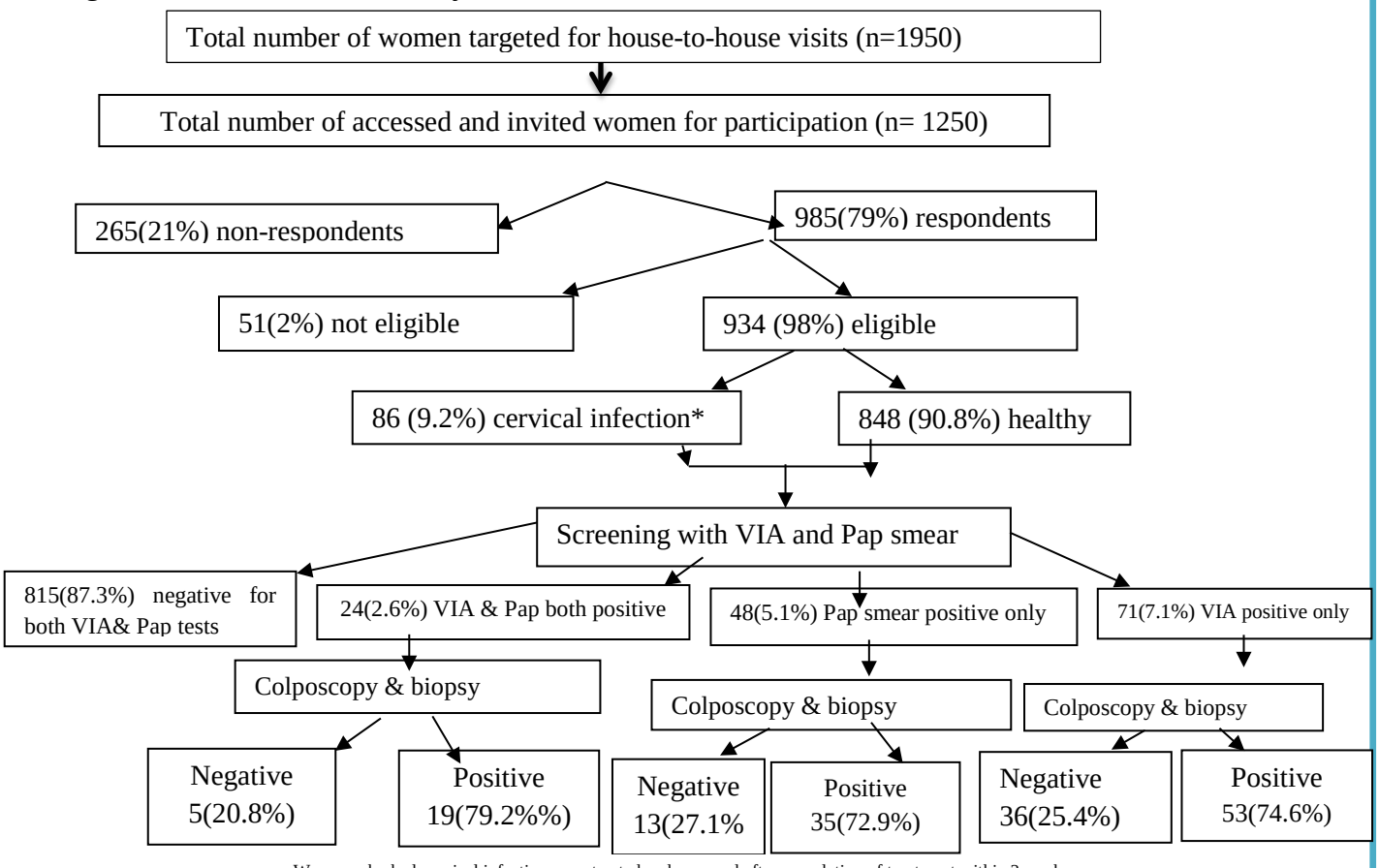
The overall mean ±SD age of participating women was 34.9±7.6; it was 34.8±7.6 years for screened positive women and 32.8±7.6 for screened negative women. The mean age of sexual initiation was 20.1±2.1 for all women in the study sample. It was 20.3±2 for screened positive women and 19.8±2.1 for screened negative women. Number of deliveries ranged from one to seven live births with mean of about two births for the study sample. The main education level was formal basic school (73.5%). Majority (63.7%) of participating women were principally housewives. The residence area was urban for 63.8% and rural for 36.2%of women. About 90% of screened women had had genital mutilation. Preponderance of women (76%) in study sample used oral contraceptive. In terms of parity, 32.2% of the screened women were nulliparous and 64.8% were parous. About 76% of women were episiotomized during vaginal delivery (Table 14).

Before screening all women had undergone counseling and clinical assessment; 90.8% (848/934) women were observed to have a normal cervix and 9.2% (86/934) had signs of

cervical infection. They were investigated and were found to have different types of infections including chlamydia, bacterial and Candida albican. They received appropriate treatment and were screened after two weeks after the recovery from the infection.

The results of all screened women revealed that 12.7 % (119/934%) was positive, of which 7.6 % (71/934) was positive with VIA test, and 5.1 % (48/934) was positive with Pap smear (Table 14). There was an overlap between VIA and Pap smear in positive results of 20.2 % (24/119) of all screened positive women. Comparing characteristics of the screened positive women between VIA test and Pap smear revealed that there was a highly significant difference in age of women who were tested positive by VIA and Pap smear (32.3±6.7 versus 38.3±6.3; p <0.0001) respectively. There was also a significant difference between positive results of VIA and Pap smear in relation to women who had had female genital mutilation (93% versus 79.2%; p< 0.0001).

Figure 9: Flow chart of the study



*Women who had cervical infection were treated and screened after completion of treatment within 2 weeks

Table 12: Characteristics of the participants screened with both VIA and Pap smear (n=934)

Variables	Total (n %)	Screening		
		Negative	Positive	*P value*
Total participants	934	815 (87.3%)	119 (12.7%)	
Age (mean±SD)	34.9±7.6	34.8±7.6	32.8±7.6	0.01
Age(year) of sex initiation	20.1±2.1	20.3±2.0	19.8±1.9	0.01
No. of delivery	1.81±2.3	1.71±1.8	1.92±2.1	0.27
Education level				
No formal education	247 (26.4%)	216 (26.5%)	31 (26%)	0.01
Formal school	687 (73.6%)	599 (73.5%)	88 (74%)	
Employment				
Employed	338 (36.2%)	286 (35.1%)	52 (43.7%)	0.06
Unemployed	596 (63.8%)	529 (64.9%)	67 (56.3%)	
Residence				
Rural	354 (37.9%)	314 (38.5%)	40 (33.6%)	0.30
Urban	580 (62.1%)	501 (61.5%)	79 (66.4%)	
Female Genital Mutilation				
Yes	836 (89.5%)	732 (89.8%)	104 (87.4%)	0.12
No	98 (10.4%)	83 (10.2%)	15 (12.6%)	
Contraceptive use				
Yes	710 (76%)	620 (76.1%)	90 (10.4%)	0.91
No	224 (24%)	195 (23.9%)	29 (3.1%)	
Delivery type				
Nulliparous	329 (35.2%)	281 (34.5%)	48 (403%)	0.21
Parous	605 (64.8%)	534 (65.5%)	71 (59.7%)	
Episiotomy				
Yes	710 (76%)	624 (76.6%)	86 (72.3%)	0.31
No	224 (24%)	191 (23.4%)	33 (27.7%)	
Cervical Infection				
No Infection	848 (90.8%)	779 (95.6%)	69 (58%)	0.00
Infected	86 (9.2%)	36 (4.4%)	50 (42%)	

There was a significant difference between women who had cervical infection with positive VIA test and women with cervical infection and positive Pap smear test (33.9% versus 54.2%; p=0.04) (Table 13).The results of histopathology revealed that 88/119 (73.9%) confirmed positive of which 53/71 (74.6%) had VIA positive and 35 (72.9%) had positive Pap smear. Moreover, classification of the positive specimens showed that 21 (31.8%), 26 (39.4%) and 28 (42.2%) were CIN1, CIN2 and CIN3 respectively. The histopathological result of the confirmed cases differed based on the screening test; CIN1 was 11.3% versus 25.7%; CIN2 was 28.3% versus 48.6%; and CIN3 was 52.8% versus 71.4% for VIA and Pap smear respectively (Table

(15). Overlapping positive cases by both with VIA and Pap smear were 24 of which 52.6%, 31.6%, 10.5%, and 5.3% were CIN1, CIN2, CIN3 and stage 1 respectively.

Table 13: Comparison between positive VIA and positive Pap smear tests and characteristics of participants (n=934)

		Screening Test*		
	Total Positive	VIA	Pap Smear	*P value*
	N=119 (12.7%)	71 (7.6%)	48(5.1%)	0.004
Continuous Variables	(Mean±SD)	(Mean±SD)	(Mean±SD)	*P value*
Age in years	32.8±7.6	32.3±6.7	38.3±6.3	0.0001
Age (mean ±SD) of sex initiation in year	19.8±1.9	19.8±1.9	20.3±2.0	0.1
No. of deliveries (mean ±SD)	1.92±2.1	1.71±2	1.92±2.3	0.5
Categorical Variables	*Total N (%)*	*N (%)*	*N (%)*	*P value*
Education level				
No formal education	31 (26.0%)	19 (26.8%)	12 (25%)	0.9
Formal education	88 (55.5%)	52 (54.9%)	36 (65.3%)	
Employment				
Employed	52 (43.7%)	29 (40.8%)	23 (47.9%)	0.5
Unemployed	67 (56.3%)	42 (59.1%)	25 (52.1%)	
Residence				
Rural	40 (33.6%)	23 (32.4%)	17 (35.4%)	0.8
Urban	79 (66.4%)	48 (67.6%)	31 (64.6%)	
Female genital mutilation				
Yes	104 (87.4%)	66 (93%)	38 (79.2%)	0.05
No	15 (12.6%)	5 (7%)	10 (20.8%)	
Contraceptive use				
Yes	90 (10.4%)	55 (77.5%)	35 (72.9%)	0.08
No	29(3.1%)	16 (22.5%)	13 (27.1%)	
Delivery type				
Nulliparous	46 (38.7%)	30 (42.3%)	16 (33.3%)	0.9
Parous	73 (59.7%)	41 (57.7%)	32 (66.7%)	
Episiotomy				
Yes	86 (72.3%)	55 (77.5%)	31 (64.6%)	0.8
No	33 (27.7%)	16 (22.5%)	17 (35.4%)	
Cervical infection				
No infection	69 (58%)	47 (66.1%)	22 (45.8%)	0.04
Infected	50 (42%)	24 (33.9%)	26 (54.2%)	

Table 14: Comparison between VIA and Pap positive total women (n=119) undergone colposcopy and histopathology

	Total(N)	VIA Test No (%)	Pap smear No (%)	*P value*	Overlap VIA/Pap positive
Total screened positive	119	71 (7.5)	48 (5.1)	0.004	24
Total confirmed by colposcopy and biopsy	88(73.9%)	53 (74.6)	35 (72.9)	0.05	19(79.2%)
Classification of confirmed positive					
CIN1	12(13.6%)	4 (7.5)	8 (22.9)	0.2*	10(52.6%)
CIN2	26(29.4%)	14 (26.4)	12 (34.3)	0.7	6(31.6%)
CIN3	28(31.8%)	16 (30.2)	12 (34.2)	0.4	2(10.5%)
Stage1	22(25%)	19 (35.8)	3 (8.6)	0.001*	1(5.3%)

* Yates Chi-square test and P value

5. 5. Comparison of the performance of VIA and Pap smear tests *(definitive study)*

Since there is verification bias due to women who were screened negative by both screening tests didn't received confirmatory examination by colposcopy and biopsy. The performance of screening tests was estimated by detection rate for high grade lesions (CIN2, CIN3 and stage 1). Comparison of the performance of VIA and Pap in terms of sensitivity, specificity, positive and negative predictive values were calculated by construction of 2x2 tables (15, 16)

Table 15: Performance VIA test total screened women (N=934)

		Colposcopy + biopsy		
		Positive	Negative	Total
VIA Test	Positive	49	22	71
	Negative	39	824	863
	Total	88	846	934

Sensitivity = 55.7%, Specificity =97.4%, Positive Predictive Value=69.0%, Negative Predictive Value =95.5%

Table 16: Performance Pap smear test total screened women (N=934)

		Colposcopy + biopsy		
		Positive	Negative	Total
Pap smear	Positive	35	13	48
	Negative	53	833	886
	Total	88	846	934

Sensitivity = 30.8%, Specificity =97.5%, Positive Predictive Value =56.3%, Negative Predictive Value = 93.1%

There was variation in performance of screening tests which were used in this study. VIA was higher than Pap smear in sensitivity but there was no difference in specificity between the two tests. VIA had lower positive predictive value compared to Pap smear but it has higher negative predictive value than Pap smear (Table 18).

Table 18: Comparison of VIA and Pap smear test performance (n= 934 women)

	VIA Test	95% CI	Pap smear	95% CI
Sensitivity	55.7	(44.7 - 66.3)	30.7	(21.3-41.2)
Specificity	97.4	(96.1 - 98.4)	97.5	(96.2- 98.5)
Positive predictive value	69.0	(56.9-79.5)	97.5	(96.2- 98.5)
Negative predictive value	95.5	(93.9 - 96.8)	56.3	(41.2 - 70.5)

To assess the performance of combined VIA/Pap tests, series and parallel were used to calculate sensitivity and specificity as follows:

	Sensitivity	Specificity
Parallel	Sensitivity A + Sensitivity B - Sensitivity A x Sensitivity B	Specificity A x Specificity B
	Sensitivity VIA+ Sensitivity Pap – Sensitivity VIA x Sensitivity Pap = 55.7% + 30.8% - 55.7% x 30.8%=**69.4%**	Specificity VIA x Specificity Pap = 55.7 %x 30.8% =**95%**

Parallel result of sensitivity of VIA/Pap test was 69.4 % and parallel specificity of both test was 95%. Combined tests have high sensitivity compared to each test individually, but parallel specificity of both tests is lower compared to specificity of each test individually.

5.6. Physicians' knowledge and practice of cervical cancer (*Physicians survey*)

Total number of invited participants was 230 physicians; 171 of them completed and returned the questionnaire with the response rate of 74.3%. Eleven questionnaires 6.4% (11/171) were excluded from the analysis due to incomplete answers. The total number of the analyzed questionnaires was 160 (69.6%), of which 49 (30.6%) were answered by obstetricians/gynecologists and 111(69.4%) by general practitioners. Table 20 shows the characteristics of the participants. The mean age of participants was 43.8 years and the mean work experience was 6.3 years. Gender distribution: 61.3% were males and 38.7% were females. Participants' education institutions: 70% of them had graduated from public university; general practitioners had graduated more frequently from private universities than obstetricians/gynecologists (35.1% versus 18.7%; p= 0.03). About 66.9% of the participants were employed by governmental sector and 71.3% worked in full time jobs.

Majority of physicians (71.9%) spent their time in antenatal care and very little time was spent for gynecological practice (15%); the rest was spent in gynecological and obstetrics operations. Obstetricians/gynecologists more often than general practitioners (77.6% versus 21.9%; $p<0.0001$) stated that cervical cancer diagnosis and treatment was part of their work.

Expectedly, in the practice of cervical cancer diagnosis and treatment, obstetricians/gynecologists performed more gynecological procedures than general practitioners. There was a significant difference between obstetricians/gynecologists and general practitioners in Pap smear practice (36.7% versus 5.4%; p=0.01), colposcopy (69.4% versus 13.5%; p=0.007) and hysterectomy (44.9% versus 9%; 0.03). Although there was a difference in cryotherapy practice between the two groups (36.7% versus 9%), this difference was not statistically significant (p=0.1). However, there was a highly significant difference between obstetricians/gynecologists and general practitioners who did not perform any

gynecological procedure during the last 6 weeks (12.2% versus 49.5%; $p < 0.0001$) respectively.

Participants were asked about the source of knowledge on cervical cancer screening. Remarkably, 72.5% and 54.4% of participants stated that cervical cancer screening methods were not part of their education and training and that it was not included in clinical practice at their working health care setting either. Since the focus of the study was knowledge and practice of cervical cancer screening, the participants were asked about their perception of cervical cancer in Sudan. About 83% of the participants perceived cervical cancer as a major health problem in Sudan. Sixty three percent of the participants stated that the solution of this problem can be managed by diagnosis and treatment and 80.1% responded that the problem can be managed by initiation of cervical cancer screening programme.

The response of participants to the questions about cervical cancer prevention and screening methods varied between the two groups of physicians. Only 43.1% of the participants answered that human papillomavirus vaccination is a preventive method for cervical cancer, and 47.5% of participants stated that HPV vaccination is not HPV vaccination ultimate method for prevention of cervical cancer. There was a significant difference between obstetricians/gynecologists and general practitioners in the knowledge of HPV vaccine (65.3% versus 33.3%; $p<0.0001$). About screening methods, 84.4%, 53.1%, 47.5% and 13.8% of the participants knew Pap smear, cytology-based, HPV and VIA as screening methods for cervical cancer respectively. There were significant differences between the two groups of health professionals in respect to knowledge of each method of the cervical screening.

Table19: Physicians' practice of cervical cancer in Khartoum State, Sudan

		Physicians		
		Obstetrician and Gynecologist	General Practitioner	
		N (%)	N (%)	
Characteristics	Total(160)	49 (30.6%)	111(69.4%)	*P* value
Major clinical practice				
Antenatal care	115(71.9%)	31(63.3%)	84(75.7%)	0.1
Gynecologic clinic	32(15%)	10(20.4%)	22(19.8%)	
Obstetric /gynecologic operations	13(8.1%)	8(16.3%)	5(4.5%)	
Diagnosis of cervical cancer in practice				
Frequently	68(42.5%)	32(65.3%)	36(32.4%)	0.0002
Sometimes	56(35%)	11(22.5%)	45(40.5%)	
Rarely	36(22.5)	6(12.2%)	30(27.1%)	
Gynecological procedure done during last 3months				
Pap smear	24(15%)	18(36.7%)	6(5.4%)	0.01
Cone biopsy	46(28.8%)	32(65.3%)	14(12.6%)	0.007
Colposcopy	49(30.6)	34(69.4%)	15(13.5%)	0.006
Cryotherapy	29(26.1%)	18(36.7%)	11(9.9%)	0.1
Hysterectomy	32(20%)	22(44.9%)	10(9%)	0.03
Dilatation and curettage (for abortion)	61(54.9%)	6(12.2%)	55(49.5%)	0.001

Table 20: Physicians' knowledge about cervical cancer screening and prevention methods in Khartoum State, Sudan

		Physicians		
		Obstetricians/ Gynecologists	General Practitioner	
Variable	Total (160)	49(30.6%)	111(69.4%)	P value
Cervical cancer screening methods was part of medical education				
No	44(27.5%)	21(43.8%)	23(20.7%)	0.003
Yes	116(72.5%)	28(56.2)	88(79.3%)	
Sources of knowledge in continuous medical education				
Textbooks	74(46.3%)	25(51%)	49(44.1%)	0.03
Journal	42(26.2%)	17(34.7%)	25(22.5%)	
Courses, conferences & symposiums	44(27.5%)	7(14.3%)	37(33.3%)	
Knowledge all existed risk factors for cervical cancer				
Yes	101(63.2%)	41(83.7%)	60(54.1%)	0.0007
No	59(36.8%)	8(16.3%)	51(45.9%)	
Knowledge of screening age group of screening				
Yes	129(80.6%)	41(83.6%)	78(70.3%)	0.1
No	31(19.4%)	8(16.4%)	33(29.7%)	
Knowledge of screening interval time				
Yes	100(62.5%)	40(81.6%)	59(53.2%)	0.0001
No	60(37.5%)	9(18.3%)	52(46.8%)	
Knowledge of screening methods				
Pap smear	135(84.4%)	47(95.9%)	88(79.3%)	0.0001
VIA	138(86.2%)	35(71.4%)	103(92.8%)	0.002
HPV	76(47.5%)	45(91.8%)	31(27.9%)	0.0001
Perceived cervical cancer as major health problem				
Yes	132 (82.5%)	42(83.7%)	90(81.1%)	0.6
No	28(17.5%)	7(14.3%)	21(18.9%)	
Cervical cancer problem can be solved diagnosis and treatment				
No	100(62.5%)	40(81.6%)	60(54.1%)	0.001
Yes	60(37.5%)	9(18.4%)	51(49.9%)	
Cervical cancer problem can be solved by screening program				
Yes	129(80.6%)	38(77.6%)	35(21.9%)	0.0001
No	31(19.4%)	11(22.4%)	76(78.1%)	
Cervical cancer problem can be solved by vaccination of women against HPV				
Yes	69(43.1%)	32(65.3%)	37(33.3%)	0.001
No	91(56.9%)	17(34.7%)	65(58.6%)	

6. Discussion

In this study about cervical cancer risk factors, feasibility and acceptability of VIA screening method in Khartoum state, Sudan, findings showed that women, who have uterine cervix laceration, assisted vaginal delivery, female genital mutilation, or episiotomy, are at an increased risk of being positive with VIA test. Women with cervical cancer who are elderly, not covered by health insurance, of African ethnicity, and living in a rural area are more likely to be diagnosed at an advanced stage of cervical cancer in Sudan. Use of VIA and Pap smear screening tests identified 12.7% positive women, VIA significantly detected more positive women than Pap smear (7.6% versus 5.1%; p=0.004). VIA has higher sensitivity and lower specificity compared to Pap smear. Also VIA was acceptable to majority of screened women and surveyed physicians have adequate knowledge on cervical cancer and screening methods.

The overall findings indicate that VIA is useful for screening of cervical cancer in primary health care settings in the study area; however, positive results need to be confirmed by colposcopy and biopsy. It also showed that VIA is a feasible and acceptable cervical cancer screening method in a primary health care setting in the Sudanese context.

6.1. Risk factors of cervical cancer and feasibility of VIA screening in Khartoum State in Sudan

In this pilot study of 100 women in Khartoum State, Sudan, 16% of women had a positive VIA result. The major observed risk factors were uterine cervix laceration, assisted vaginal delivery, female genital mutilation, and episiotomy. The VIA prevalence of 16% was the same as in Nigeria; but higher than in Kenya, Ghana (14%), and Latin America (12%); and lower than in South Africa and Zimbabwe, where the VIA test was positive in 26% of the study population[103, 106-110] The results strongly suggest that incidents causing trauma to the

uterine cervix are risk factors for VIA positive cervical cancer in Khartoum State. This was true for women with uterine cervix laceration, genital mutilation, women who delivered vaginally, women who had had an episiotomy, women who had undergone assisted vaginal delivery, and parous women. Also, earlier episiotomy has been reported as a site for implantation and recurrence of cervical cancer in women who had cervical cancer during pregnancy and delivered vaginally. It has been reported that metaplastic changes are also influenced by the trauma and repair experienced during delivery, and increased risk of cervical carcinoma has been identified in women who are highly parous.[111-115] The results of this study revealed that uneducated and unemployed women had high risk of being VIA positive, which are consistent with previous studies that show that cervical cancer is more prevalent in low-educated and low socioeconomic status populations.[116;117] Factors such as cosmetic smoking of the lower body and partner circumcision are very common practices among the sample, which made it impossible to study them as risk factors for cervical cancer. This remains to be researched in a larger or more varied sample. The study results provide new risk factors for VIA positivity: assisted vaginal delivery, episiotomy, and female genital mutilation. Any trauma to female genital organs is a predisposing factor to infection. Episiotomy, cervical laceration, and genital mutilation are major types of iatrogenic trauma.[118] Infection with human papillomavirus is a fundamental risk factor for cervical cancer. The majority of women were episiotomized during delivery, and a higher number of pregnancies and multi-parities were reported as risk factors for positive screening test with VIA. Women with these factors were about nine times more prone to have positive VIA test than women without these factors. Previous studies found that episiotomy is risk factors for cervical cancer. [119,120] If pregnant women diagnosed with cervical cancer are treated prior to delivery and go on to have an episiotomy, the cancer cells here will undergo metastasis.[119]

This pilot study showed that over two-thirds of women approached took the VIA screening test. One explanation for this relatively high participation rate was probably the active information campaign. The screening facility was relatively easily accessed, and the examination was acceptable for most of the women. The response rate to screening was equal to that in Thailand[121] but lower than that in the Philippines, and higher than that in Ghana.[108] The test is very simple and can be used effectively by nurses after two days of training. It is very cheap, costing about US$5 per visit for a 3-year screening strategy. This study is limited by its small sample size. A large sample size is needed to clarify the nature of the observed association between cervical cancer and risk factors in these results.

6.2. Predictors of advanced stages of cervical cancer at diagnosis in Sudan

In the hospital-based survey for determining predictors of advanced stages of cervical cancer at diagnosis in Sudan, it was revealed that about 72% of registered cases of cervical cancer during year 2007 were diagnosed at advanced stage. It proved that old age, lack of health insurance, African ethnicity, and living in rural areas were independent risk factors for advanced stage of cervical cancer. The risk of advanced stage was especially high among women who had no health insurance. This finding is consistent with earlier studies on the same topic.[122-125] Women without health insurance are less likely than those with health insurance coverage to seek health care and to receive appropriate medical care services. Health insurance in Sudan has low coverage as only about 46% of total population, those employed by government and large companies are covered by health insurance. Those who are not covered by health insurance should pay for health services.

In this study almost 46% of advanced stage (stage III& IV) of all cervical cancer cases was in the age group≥55 years. Older women were less often diagnosed at early stage of cervical cancer compared younger women. This may be due to lack of seeking obstetrics and

gynecological medical care in post-menopausal period, particularly for women who live in rural areas where health care services are difficult to access. Another likely factor could be lack of awareness about susceptibility of cervical cancer.[126-128] Furthermore, a crucial factor that probably has contributed to the delay in detecting cervical cancer is poor dissemination of knowledge, information and communication by health care providers.[129-132]

This study also found that among women diagnosed as having cervical cancer, those who were of African ethnicity, were more likely to be diagnosed with advanced stage disease compared with those who were of Arabic origin. Difference among ethnic groups in stage at diagnosis of cervical cancer has been reported in several other studies. Brewer et al[131] found major ethnic differences in cervical cancer survival in Maori and Pacific women in New Zealand. They reported that the difference was almost entirely due to stage at diagnosis, indicating that ethnic differences in access to and uptake of screening and treatment of pre-malignant lesions may have been playing a major role. Brookfield et al found similar results in a study from Florida including Caucasian, African American and Hispanic women. Their study concluded that race, ethnic, and socioeconomic disparities in cervical cancer survival were explained by late-stage presentation and under-treatment. [134] In line with our results, Downs et al[135] found that African and Hispanic women as well as older women were more likely to be diagnosed with late-stage of cervical cancer. This difference is due lack of awareness, poverty and lack of health insurance, which result in an underprivileged situation in terms of access to health care services.

In this study there was a difference in urban and rural distribution between women of African and Arabic ethnicity; however, spatial distribution of African and Arabic ethnicity in Sudan in respect to hospital care is equal. Spatial disparity in access to health services exists between urban and rural area, and it is likely - though there is no clear evidence - that African ethnicity

concentrates in rural areas. In rural areas there is lack of access to health services and of health insurance coverage and this probably leads to late presentation of care seeking resulting in diagnosis of cervical cancer at advanced stages.

Single women in Sudan commonly are not sexually active; consequently they rarely seek reproductive health care and hardly have obstetrics and gynecological examination. Due to inherent social stigma about non-virginity, single and unmarried women are considered to be virgin. Unmarried women may have refused to have gynecologic examination due to fear of potential social stigma they would suffer if they had a test perceived positive.[135] Moreover, social values portraying sex outside of marriage as sinful, are often believed to contribute to gynecological diseases. Further, stigma associated with sinful behavior is frequently assumed to interfere with access to health care for those who have gynecological disease, because cervical cancer is often associated with sexually transmitted infections and prostitution.[137] Therefore the disease is often diagnosed at advanced stage in such groups of women. In this study married women were more frequently diagnosed at early stages of the disease compared to unmarried women; this may be due to more frequent obstetrics and gynecological health care they received during childbearing period.

6.3. VIA and Pap smear screening in Khartoum State, Sudan

Sudan is ranked at the top level of cervical cancer morbidity and mortality in northern African countries.[2] In these countries there are no screening programmes or the programmes are ineffectively developed and poorly organized. The majority of the screening programmes are founded on Pap smear method and attempt to imitate the excellent outcome achieved in developed countries. Nevertheless, the results of these programmes were very poor due to the lack of infrastructure, inadequate training of medical staff, poor organization, lower coverage of women at risk, no standardized quality control systems, and a lack of follow-up and

treatment of positive cases.[4] Therefore, in recent years screening with VIA has emerged as an alternative to conventional Pap smear.[104] VIA has become a promising alternative for developing countries because it is inexpensive, rapid and it requires only short training and does not need laboratory equipment. A number of earlier studies have reported that VIA has a comparable result similar to or superior to the Pap smear in the detection of cervical cancer. [138,139]

This thesis work is the first study carried out to determine the feasibility of VIA as screening method for cervical cancer in primary health care setting in Sudan. The study was preceded by a pilot study which showed that 16% of screened women were positive to VIA. The result of final study is lower compared to pilot study (7.5% versus 16%) respectively. This difference could be due to sampling variation, since the sample size in the pilot was small compared to the final study (100 versus 934). The findings of this study revealed that VIA significantly detected more positive women than Pap smear (7.6% versus 5.1%; p=0.004). There was an overlap between the Pap smear and VIA screening test in 20% of screened positive. VIA had higher sensitivity and positive predictive value than Pap smear but had lower specificity and negative predictive value respectively. VIA detected more confirmed diagnosed cases of intraepithelial cervical neoplasia (CIN) than Pap smear although the difference was statistically non-significant; however, in the confirmed diagnosed cases of invasive cervical cancer in stage 1, VIA significantly detected more cases than Pap smear (19 versus 3; p=0.001).

The VIA screening test detected 7.5% positive of cases, which was higher than that found by Nessa et al [140] in Bangladesh (4.8%) and Muwongeet al [141] in Angola (6.6%), but lower compared to that found by and Were et al in Kenya (13.9%) [142] Perkins et al[143] in Honduras

(14%), and it was much lower than that found by Cremer et al[144] (26.5%) in El Salvador and Ekalaksananan et al [145] in Thailand (38.1%).

One more essential finding concerned the total percentage of women who were tested positive by both screening tests; this was 12.7%. The percentage was significantly lower in Pap smear positive women (5.1%) than in women with a positive VIA test (7.5%), (P< 0.004). In this study the sensitivity of VIA was 55.7%, which was higher compared to the sensitivity of Pap smear which was 30.7%. It was lower to that reported by Ngoma et la[146] in Tanzania (60.6%), higher than that reported by Murillo et la[147] in Colombia (53.6%) but lower than that reported by Sahasrabuddhe et al [148] in India (80.%), Muwonge et al[149] in Angola (88.0%), and by Aggarwal et al[150] in India (98%). Sensitivity of VIA test in this study was consistent with that described by IARC multicenter study in India and Africa which found pooled VIA sensitivity of 50-96%. [151] The specificity of the VIA test in this study was 97.4%; it was higher compared to Pap smear (65%), and to that found in by Mutyaba et al[152] in Uganda (75%), Muwonge et al[153] in Angola (94.5%), but consistent to that reported by IARC screening group (44-97%).[151] There was a difference between VIA and Pap in the total number of confirmed cases of cervical intraepithelial neoplasia by colposcopy (53 versus 35) for VIA and Pap respectively. The difference was marginally significant (P=0.05).There was no significant difference in confirmed cases of CIN2, CIN3, and CIN3, between VIA and Pap smear. However, VIA detected more cases of cervical cancer in stage1 compared to Pap (19 versus 3) with statistically significant difference ($p<0.001$). The positive predictive value for VIA of this study is higher than that found by Bhatla et al[154] in Kolkata, India. In this study VIA detected more advanced cervical intraepithelial neoplasia than Pap smear; this is probably due to easy uptake of the neoplasia of acetic acid and easy visibility by naked eyed. Pap smear needs advanced experience and skills in smearing sampling technique. VIA increases detection of

premalignant lesions of the cervix and diminishes the probability of losing women before they are appropriately followed up and treated. This study suggests that VIA can be used as a screening tool in poor countries not only in primary health care setting, but also in general hospitals.

Parallel result of sensitivity and specificity of VIA and Pap test was 69.4 % 95% respectively. The parallel tests have high sensitivity compared to each test individually, but parallel specificity of both tests is lower compared to specificity of each test independently.

6.4. Physicians' knowledge and practice of cervical cancer screening

This study assessed the knowledge and practice in two groups of physicians principally involved in obstetric and gynecologic health care services in Sudan. The information was obtained from about 75% of surveyed physicians. The response rate was higher among general practitioner physicians than obstetricians/gynecologists (80.4% versus 53.5%) respectively. Majority of participants were male and this signified dominance of male gender in obstetrics and gynecology practice in Sudan. The main clinical practice of all participating physicians was antenatal care (72%). This type of practice creates prudent opportunity for cervical cancer screening in the future if it is wisely planned. The highest proportion (42.5%) of the participants stated that they frequently diagnosed cervical cancer during their practice. The remarkable finding was that 83% of surveyed physicians perceived that cervical cancer was a major health problem in Sudan. Most of the physicians (62.5%) claimed that the solution of cervical cancer can be tackled by diagnosis and treatment. Furthermore, high proportion (81%) of the participants identified that solution of cervical cancer in Sudan will be through establishment of a screening programme. Additionally, (43.1%) of the respondents replied that cervical cancer can be prevented by vaccination against human papillomavirus infection. Most likely lack of knowledge and training in vaccine use and high vaccine cost

were the major factors that prevented physicians from recommending vaccines.[156] Other factors might be related to the lack of financial assistance and insufficient role of government in vaccine promotion as has been suggested in others studies.[157,158]

More than 70% of participants stated that cervical cancer screening was not included in the education or training in the medical education. This reflects serious lack of medical education curriculum at the universities for the topic of cancer prevention and control. Schnatz et al reported that majority of physicians believed that their training was less than adequate and believed that their practice would benefit from continuing medical education courses.[159]

The study findings showed a significant difference in practice of cervical cancer diagnosis and treatment between obstetricians / gynecologists and general practitioners; the first group practiced more than the second group. This is due to their medical specialty which offers more opportunities to practice for obstetricians/gynecologists than for general practitioners. Aldrich et al reported a significant difference between obstetricians/ gynecologists and general practitioners in the use of Pap smear and colposcopy practice in diagnosis of cervical cancer in Mexico.[160] In spite of differences between the obstetricians/gynecologists and general practitioners in knowledge of cervical cancer risk factors (83.6% versus 54.1%; p=0.0004), the proportion of physicians who knew the major risk factors was relatively high (63.2%). Nevertheless, the percentage of all physicians who knew the principal risks factors for cervical cancer was low compared to Mexican survey where 80% of providers in the national survey knew that. [160] Our study finding of knowledge was higher than the findings of Kabir et al[161] in Niger and Mutyaba et al[162] in Uganda, where the proportion health professionals who knew major risk factors of cervical cancer 60% and 40% respectively. Although obstetricians and gynecologists were more aware about human papillomavirus vaccine, physicians' knowledge about HPV vaccine as preventive method for cervical cancer

was generally low in this study. This indicates lack of updating in recent development in cervical cancer prevention issues. Physicians' knowledge about cervical cancer screening method varied from very high in Pap smear to very low in VIA; this may reflect deficiency in continuous medical education to provide physicians with up-dated knowledge in cervical screening method like VIA, which is the promising screening method for cervical cancer in low resource countries. [163]

7. Limitations and strengths

The limitations of this study vary from one sub-study to another, and they can be summarized in the following points:

- In the pilot study the sample was relatively small, although the response rate was high among the participants.
- Study of feasibility and acceptability was limited to screened women in the pilot study.
- In the hospital-based survey the collected data on earlier staging of cervical cancer, such as carcinoma in situ, were not held by the cancer registry, and the tumor stages were classified into broad major stages without sub-stages. In addition, invasive cervical cancer cases were reported to the cancer registry directly from secondary and tertiary care institutions, so the data on patients who were not hospitalized would not have been entered into this hospital-based registry. The extent to which unregistered cases might have differed in age, ethnicity, and geographical distribution was unknown.
- In the comparative study of VIA and Pap smear test the investigator had plans to perform screening for HPV and HIV tests corresponding with cervical cancer screening but due to insufficiency of funds these tests were not done.
- There was verification bias in comparative study of VIA and Pap smear because gold standard procedures “colposcopy and biopsy” were invasive and expensive and they were performed only to those who had positive screening test results. Therefore, sensitivity, specificity, positive and negative predictive values of screening tests VIA and Pap smear were estimated.

The strengths of the study can be elaborated as follow:

- The study methods were validated before being used in the pilot study.
- This is the first study to investigate cervical cancer in Khartoum state particularly and Sudan generally.
- Comparison of VIA and Pap smear study has relatively big sample size and its findings can be generalized to the study population in the study area.
- The study provided some evidence about new risk factors for positive VIA test which may be risks for cervical cancer if they are proved by well-designed by case- control studies in the future.
- The study used a new method of screening by VIA for cervical cancer and it found that it had high sensitivity in the detection of cervical cancer compared to Pap smear in the primary health care setting.

The study provided some evidence which can be used by decision-makers to initiate cervical cancer screening by use of VIA screening in Khartoum state.

8. Conclusion

The study showed that women, who had trauma to their cervix, such as uterine cervix laceration, assisted vaginal delivery, female genital mutilation, or episiotomy, are at an increased risk being screened positive with VIA. The results showed trauma to the cervix as being a risk factor for infection which may result in cervical cancer. This finding points to the importance of safe delivery facilities and establishing guidelines and standard operation procedures for performing assisted vaginal delivery and episiotomy in obstetrics practice. Also, abandonment of female genital mutilation can have a great effect in decreasing the incidence of cervical cancer. Training of birth attendants on safe delivery services and increasing community awareness about female genital mutilation risks can play a great role in talking of the problem. Further decision on the introduction of cervical cancer screening in Khartoum state in Sudan is critically needed.

Women with cervical cancer, who are elderly, not covered by health insurance, are of African ethnicity and live in rural area, are more likely to be diagnosed at advanced stages of cervical cancer in Sudan. These women should be targeted by cervical cancer screening, health education programme and health insurance coverage.

The results of this study showed that VIA has high sensitivity and lower specificity compared to Pap smear. Combination of VIA/Pap increased sensitivity and specificity of detection of cervical cancer. The findings of study indicate that VIA is useful for screening of cervical cancer in primary health care setting and it is also a feasible and acceptable screening method in the primary health care setting in Khartoum State in Sudan.

The study findings showed that obstetricians /gynecologists have more adequate knowledge on cervical cancer screening methods than general practitioners. More efforts are needed to

develop and to adapt new strategies for promotion and improvement of cancer prevention methods in continuous medical education for general practitioners and in medical education curriculum at medical schools in Sudan.

9. Recommendations

Based on the study findings, the following recommendations can be used to initiate and establish preventive services for cervical cancer in Khartoum state, Sudan.

- Development of population-based cervical cancer screening programme.
- Integration of cervical cancer screening programme in primary health care services.
- Implement VIA as primary screening test for cervical cancer in Sudan.
- Benefit from physicians' knowledge and practice about cervical cancer in the development of cervical cancer preventive services in Khartoum state.
- Increase community awareness about health consequences of female genital mutilation.
- Endorsement of strict legislation for prohibition of female circumcision practice.
- Increase training of birth attendants on safe delivery.
- Increase health insurance coverage for poor population in rural areas.
- Development of research agenda on the determinants of cervical cancer and interventional methods.
- Case-control design is definitely needed to address risk factors of cervical cancer, specifically female genital mutilation, episiotomy and assisted delivery.

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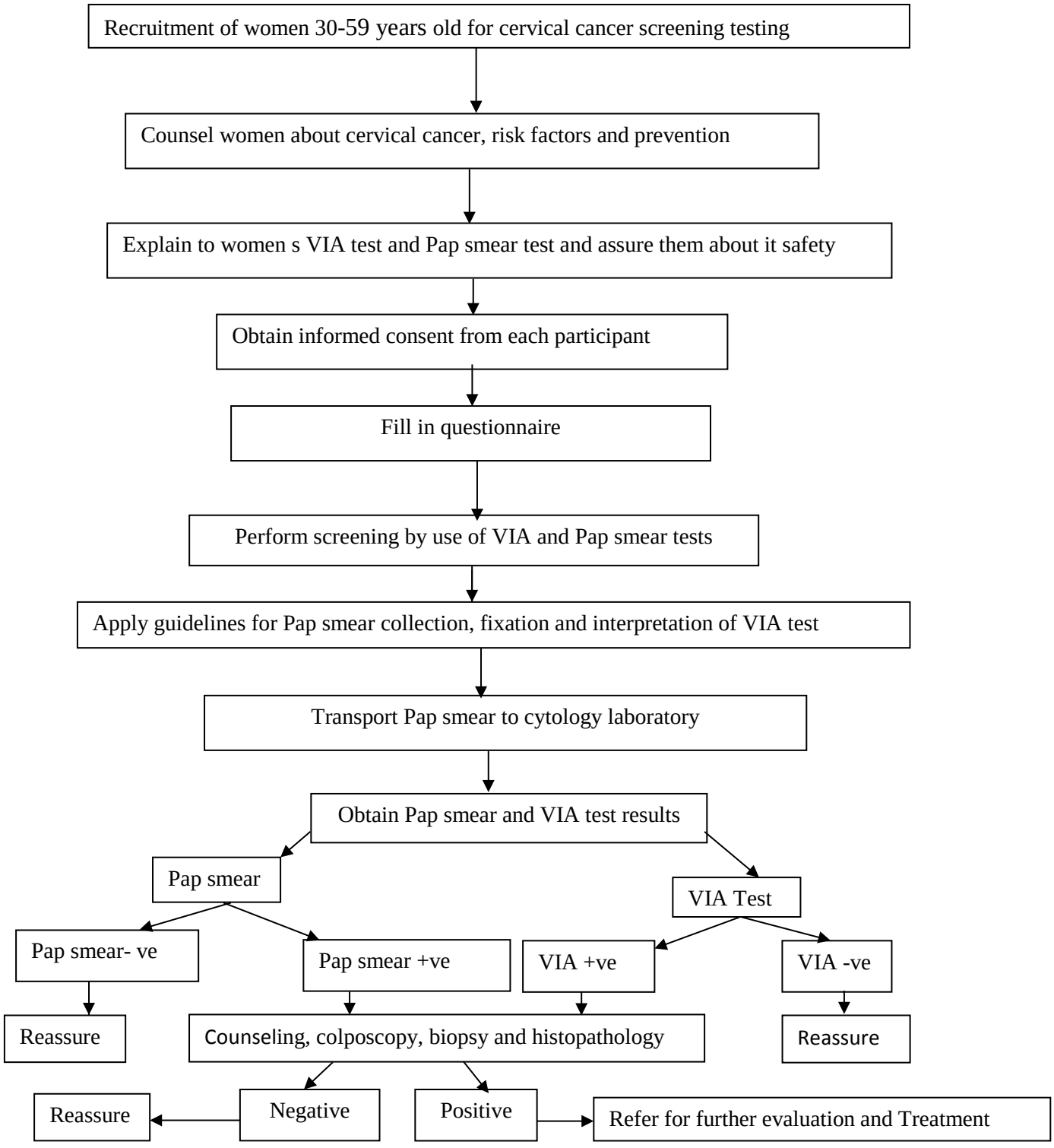
Appendices

11. Appendices

11.1. Study flow Chart



11.2. Informed Consent

Informed Consent Form

The Nurse/midwife explained to me in detailed about visual inspection with use of acetic acid test for early detection and prevention of cancer in the neck of my uterine cervix (womb). I understand that the surface of my cervix will be visually inspected by use of speculum and two specimens will be taken from my womb for laboratory investigation. I understand these procedures are generally harmless, but it may cause mild irritation and discomfort which will subside immediately.

I understand that, if the test is positive, other tests such as magnified inspection of the cervix with instrument called colposcopy and examination of sample of tissue in my cervix (biopsy) may be recommended before treatment is provided. I have been informed that treatment by medicines or cryotherapy (destroying the diseased portion of the cervix by and ice-cold metal prope) or removing the diseased portion by minor or major surgery and treatment with X-Ray may be required, in event of any abnormality (infection or precancerous or cancer or complications) being detected.

I hereby express my willingness to undergo the above tests and treatment.

Name:_____

Signature:_____ Date _____

Address: Street_____ House. No. _____

Tel_____ Mobile_____

E-mail_____

11.3. Questionnaire 1

Cervical cancer risk factors and feasibility of VIA screening among women in Khartoum State, Sudan.

Investigator: Dr. Ahmed Ibrahim. Unit for Health Promotion Research, Institute of Public Health,

University of Southern Denmark, Esbjerg, Denmark

Contact in Sudan: Mobile 0912954472.E-mail: aibrahim@health.sdu.dk

Serial No ///

Date / / /

Clinic No

Interviewer

Age in years

Educational level

1-None

2- Primary

2- Intermediate

3- Secondary

4- College

5-Graduate

6- Postgraduate

Marital status:

1- Single

2-Married

3-Divorced

4- Widow

Age at marriage or first had sexual intercourse **Years**

Last menstrual period

1- <week

2-one –two weeks

3-three- four weeks

4- More than one month

5- Less than 12 month,

6- More 12 months

Number of pregnancies

Number of miscarriages

Are you circumcised?

1-Yes

2- No

Was your husband(s) was circumcised

1- Yes ☐

2- No ☐

Do you used wood-smoking as cosmetic?

Yes ☐

No ☐

For how long have you uses wood-smoking ☐

as cosmetic?

How frequently do you use smoking as cosmetic:

1- Every week ☐

2- Every 2-3 weeks ☐

3. Every month ☐

4- More than 1 month ☐

Do you have?

1. Urogenital tract infection ☐
2. History of STIs (STDS) ☐
3. Excessive vaginal discharge ☐
4. Itching on external anogenitalia ☐
5. Ulcer on external anogenitalia ☐
6. Lower abdominal pain ☐
7. Lower backache ☐
8. Pain during sexual intercourse ☐

9. Bleeding after sexual intercourse ☐

10. Intermenstrual bleeding ☐

Do you have multiple sexual partners?

1- Yes ☐

2- No ☐

11.4. Letter to Physicians

Dear Physician

Subject: Survey on knowledge and practice of cervical cancer screening in Khartoum State, Sudan

I'm very pleased to write to you to ask your crucial participation in in a survey about knowledge and practice of cervical cancer screening. This survey aims to improve cervical cancer control and prevention services in Sudan. Here you will find the questionnaire of the survey which contains three parts the first part about socio-demographic information, second part about source of knowledge in cervical cancer, practice of cervical cancer cervical cancer risk factors of, screening method and prevention method.

These questions are very simple and it takes approximately 15 - 20 minutes to finish it. I do appreciate you participation in this survey, and thank you so much for your precious time that you spent in answering the questionnaire.

Best regards,

Ahmed Ibrahim. MD, MPH, FCM

Unit for Health Promotion Research

University of Southern Denmark

Tel: Sudan +249 12941772, Denmark +45 6550 4214

E-mail: aibrahim@health.sdu.dk

11.5. Self –administrated questionnaire

Survey on knowledge and practice of cervical cancer screening in Khartoum State, Sudan

Dear doctor, please choose the appropriate answer

Dear doctor, please choose the appropriate answer

1. Age: ----------Year
2. Gender: 1. Male 2. Female
3. Job title: 1. General practitioners 2. Obstetrician/gynecologist
4. Graduate Institution: 1. Public University 2. Provide university
5. Type of working institution: 1. Governmental sector 2. Provide sector
6. Type of employment: 1. Full time 2. Part time
7. Period of experience ----------Year
8. Does the working institution provide health care insurance services? 1. Yes 2. No
9. Do you provide cervical cancer screening for your patients? 1. Yes 2. No
10. Was cervical screening is part of your medical education 1. Yes 2. No
11. Is cervical screening included in your practice 1. Yes 2. No
12. Which gynecological procedure done during the last 6 weeks?

i. Pap smear ii. Cone biopsy iii. Colposcopy

iv. Cryotherapy v. Hysterectomy vi. None

13. Do think that cervical cancer is a main health problem in Sudan? 1. Yes 2. No 3. I don't
14. How do you perceive cervical cancer problem in Sudan? 1. Minor health problem 2. Major health problem
15. Do you use to see cervical cancer cases in your clinical practice? 1. Yes 2. No
16. If yes, how frequently? 1. Usually 2. Sometimes 3. Rarely
17. Do you think that launch of screening program for cervical cancer is essential? 1. Yes 2. No 3. I don't no
18. Sources of Knowledge in Continuous Medical Education: 1. Textbooks 2. Journal 3. Internet
19. Age is risk factor: 1. Yes 2. No 3. I'm not sure
20. Genetic: Family history of cervical cancer: 1. Yes 2. No 3. I'm not sure
21. Early sexual initiation: 1. Yes 2. No 3. I'm not sure

22. Number of sexual partners: 1. Yes 2. No 3. I’m not sure

23. Bacterial infection: 1. Yes 2. No 3. I’m not sure

24. Human Papillomavirus infection: 1. Yes 2. No 3. I’m not sure

25. Chlamydia infection: a. Yes 1. No 2. I’m not sure

26. Cervical tear: 1. Yes 2. No 3. I’m not sure

27. Smoking: a. Yes b. No c. I’m not sure

28. Contraceptive use: 1. Yes 2. No 3. I’m not sure

29 Is HPV vaccination ultimate method for prevention of cervical cancer? 1. Yes 2. No. 3. I’m not sure

11.6. Laboratory Request From

Laboratory Request From

Serial No: __ __ __ __

Date __ / _ _/ ____Primary Health Care_____

1. Patient ’s Serial No:__ _____
2. Age ___ year
3. LMP:___ day / ____ week
4. 4.Last Pregnancy ____ Month/ ___ Years

5. Contraceptive pill Yes ☐No ☐

6. Hormone therapy Yes ☐No ☐

7. Type of specimen: Pap smear☐ cervical swap☐

8. Required Test: Cytology☐

Name of Investigator: _____ Signature _____

Pap smear

Description	**CIN Grading**	
Normal	Normal	
Atypical Reactive or Neoplastic	Atypical	
Mild Dysplasia	CIN I	
Moderate Dysplasia	CIN II	
Severe Dysplasia	CIN III	
Carcinoma in-situ	CIS	
Invasive Cancer	Invasive Cancer	

Comments:

_____
_____
_____
_____
_____
_____
_____

Name of Pathologist _____Signature_____

Date: _____

11.7. Permission for inclusion of original publications from publisher

Dear Ahmed

Thank you for your enquiry regarding permission to include your published papers as part of your PhD thesis.

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11.8. Approval of Research by ethics committee

وزارة الصحة - ولاية الخرطوم
MINISTRY OF HEALTH KHARTOUM STATE
الإدارة العامة للرعاية الصحية الأولية
DIRECTORATE GENERAL OF PRIMARY HEALTH CARE
DIRECTOR OFFICE

Date: 25/12/ 2009

Approval of PhD Research

This to certify that the Research Ethics Committee at Directorate General of Primary Health Care, Ministry of Health, Khartoum State, has approved the research which will be conducted by Dr. Ahmed Ibrahim , PhD student at Faculty of Health Sciences, University of Southern Denmark.

- The research title: Cervical cancer, risk factors and feasibility of screening with used of visual inspection with acetic acid in Khartoum State Sudan.
- Research setting: Primary Health Care centers in Khartoum State
- Target population: Married women
- Research period: December 2009 to December 2010.

The researcher should restrictedly follow the code of ethics on human research during his research and he has to preserve and handle the participants' information with confidentially and provide to them optimal level of care. Any violation to research ethics will result in termination of research and expose the researcher to legal accountability.

Abdulgadir A. Basheer MD.MPH

Chairman of Research Ethics Committee

Appendix 11.9. Publications

Publications (1-3) were reprinted and included in the thesis with the permission from publisher, Dove Medical Press Ltd (Appendix No.11.7)