AN ANALYSIS OF MORPHOLOGICAL AND MORPHOMETRICAL PARAMETERS IN ENDOMETRIAL CARCINOMA

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ABSTRACT

Histopathologic evaluation plays a major role in the prognosis and treatment of endometrial carcinoma. This when supplemented with morphometry could provide important information the advantages being objectivity and reproducibility

Methods: 47 cases of Stage 1 endometrial carcinoma were reviewed over a period of twelve years to analyse the morphological and morphometric parameters and correlate them with prognosis. Of these, 17 patients were premenopausal and 30 were postmenopausal. Only seven patients were below 40 years of age.

Results : Based on morphological analysis, 12 patients had well differentiated tumours, 22 had moderately differentiated tumours while 12 belonged to the poorly differentiated group. Among the nuclear features, nuclear area and perimeter showed significant difference between the different histological grades, the former being more significant than the latter. **Conclusions:** Hence the application of nuclear morphometry to diagnosis and tumour grading could lead to better evaluation of the tumour and aid in predicting the prognosis. In women below 40 years, there is a danger of well differentiated carcinomas being diagnosed as hyperplasia as carcinoma is rare in this age group. The application of nuclear morphometry, which is both objective and reproducible, could aid in proper diagnosis of such tumours and thus lead to earlier detection and control of endometrial carcinoma.

In troduction:

Endometrial carcinoma is the most common gynaecological malignancy next to cervical cancer in developing countries. Histopathological evaluation plays a major role in subdividing endometrial carcinomas into treatment groups. WHO has recommended endometrial carcinomas to be graded into well differentiated, moderately differentiated and poorly differentiated based mainly on the architectural pattern of glands and secondarily on cytological and nuclear appearances¹. Though architectural and nuclear grading generally correspond, sometimes they are at variance in which case the nuclear grade is the more reliable indicator of tumour prognosis. In well differentiated examples of adenocarcinoma, both architectural and nuclear differentiation may be so good that the tumour appears to satisfy none of the criteria for such a diagnosis and is difficult to distinguish as a carcinoma. Hence the application of nuclear morphometry for diagnosis and tumour grading overcomes the limitation of observer error in subjective assessment of the histological grade which may not always be reproducible.

Among the prognostic determinants other than grading are age, race, symptoms, stage, depth of myometrial invasion, DNA ploidy and receptor status. Various studies have linked all these parameters with survival,^{2,3,4} but little data is available relating nuclear morphometry and prognosis in an Indian set up.

The present study was designed to evaluate the diagnostic and prognostic value of clinical, histopathological and morphometric features. These were assessed by light microscopy and eyepiece micrometry in Stage 1 endometrial adenocarcinoma.

MATERIAL AND METHODS

<u>Material</u> :

88 cases of endometrial carcinoma over a period of 12 years (from 1984 - 1997) were seen in the department of Pathology. Among them 47 cases of Stage 1 carcinoma where the tumour was confined to the corpus including isthmus were considered for the study which comprised 53.4% of the total cases. Staging was done according to the jointly agreed scheme of FIGO, the International Union against cancer (UICC) and the American Joint Committee for cancer staging and end result reporting.⁵ The clinical details including treatment details were obtained from the archives of the department and the medical records department and were tabulated depending on age group and menopausal status.

Methods :

Haematoxylin and eosin stained sections were used to study the basic morphology of the neoplasm and for grading. In the histopathological evaluation three different steps were used. At low magnification (x250) tissue architecture features were assessed. At higher magnification (x400) nuclear size and shape were evaluated for grading. Grading was performed in compliance with the system of WHO and FIGO. The nuclear features were measured using eyepiece micrometry which can be fitted to a light microscope.

The long axis and short axis of the nucleus were measured in 50 nuclei after selecting areas containing largest and atypical nuclei in 10 adjacent fields in high power objective. The nuclei selected were clearly visible, not artificially deformed and had intact nuclear membrane and chromatin. The nuclear area and perimeter were calculated using the formula π ab and $2\pi \sqrt{((a^2 + b^2)/2)}$ respectively using a computer program. For each specimen, the arithmetic mean and standard deviation of these parameters were calculated. Thus for nuclear morphometry, nuclear area, perimeter, longest axis, shortest axis, nuclear axes ratio (longest \div shortest axis) were assessed in addition to shape.

To find the number of nuclei to be measured, these features were re-measured in the same nuclei and the 95% limits based on inter-observer variation was assessed. For the features mentioned, 25 to 40 nuclei were sufficient to have cumulative average within the 95% limits. To be on the safe side, 50 nuclei were measured in each case. Mitosis were observed, but were not quantified and hence mitotic activity index was not calculated.

Statistical analysis was done using analysis of variance method (ANOVA). The results of nuclear morphometry were tabulated against histological grade to observe whether any significant difference was present. The treatment and survival data were then correlated with histological features and nuclear morphometry.

<u>RESULTS</u>

The total number of patients diagnosed with endometrial carcinoma during the twelve year period (January 1984 to January 1997) was 88. Among them, 47 were diagnosed to have Stage 1 disease prior to treatment. The rest were in Stage 2 or had only curettage performed for diagnosis and no staging or follow up was available. The mean age of the patients was 53.70 + 11.46 years. Seven were below the age of 40 years with a mean age of 33.8 + 4.6 years. Using the criteria of menstrual history, 17 patients were in the premenopausal group with a mean age of 42 + 4.1 years. (Refer figure 1)

A review of the presenting symptoms indicated that all post-menopausal patients presented with post-menopausal bleeding. The remaining women presented with irregular bleeding (23%), abdominal pain (8.5%) and other symptoms (4.25%). No significant difference was observed between the two groups with regard to the incidence of obesity, hypertension or diabetes mellitus. The seven patients who were below forty years presented with menorrhagia or irregular periods. One of them in this group had associated diabetes.

Assessment of grading based on the architectural pattern resulted in 12 patients with tumours in the well differentiated grade (Grade 1), 22 in the moderately differentiated (Grade 2) and 13 in the poorly differentiated (Grade 3) group. Nuclear grading performed independently generally showed good correlation, except in two cases where the nuclear grading was lower than the architectural grading. Calculation of nuclear area and perimeter showed a significant difference between the three grades (Refer table 1), the difference being more significant with the nuclear area than perimeter. The premenopausal group had a higher incidence of well differentiated (6/17) and moderately differentiated (9/17) carcinoma than the post-menopausal group (well differentiated - 7/30; moderately differentiated - 14/30). None of the patients below the age of 40 years had Grade 3 carcinoma (Refer figure 1).

Depth of myometrial invasion was measured independent of grade and was classified as superficial or deep. 25 cases had myometrial invasion of which only two could be classified as deep. Both these cases belonged to the poorly differentiated group and had in addition vascular invasion. Necrosis and/or lymphoplasmocytic response were observed only in well and moderately differentiated adenocarcinoma (4.8%) Concomitant endometrial hyperplasia was seen in four cases, of which two were seen in association with moderately differentiated grade. Other associated conditions observed were leiomyoma, polyps and adenomyosis.

Treatment consisted of total abdominal hysterectomy with bilateral salpingooophorectomy in all patients. Adjuvant radiation therapy was done when indicated. Status of the patients was evaluated by repeated cytological examination or by recurrence of symptoms. Of the 47 patients, twenty eight were alive and well and these patients had tumours of histological grade 1 and 2. The five patients who died of the disease had poorly differentiated or grade 3 tumours. Two patients with grade 2 tumours died due to unrelated causes and twelve patients were lost to follow up after surgery.

DISCUSSION

In an ideal situation, a tumour from a patient would be subjected to a wide range of laboratory investigations including histopathological evaluation, a panel of antibodies, morphometry and flow cytometry. But in a limited set up, a combination of morphology and morphometry could provide important information relating to prognosis, the advantages being objectivity and reproducibility.

Each histological subtype of endometrial carcinoma has distinct biological behaviour. The two types that have favourable outcome are adenoacanthoma and adenocarcinoma which fortunately comprise 83% of all endometrial carcinomas⁶. Majority of patients present with Stage 1 disease. Better evaluation of prognostic determinants could be done by restricting the study to patients with Stage 1 disease who had a favourable subtype (adenocarcinoma) and near optimal treatment.

Age and menopausal status at the time of diagnosis were important risk determinants. As all the post-menopausal patients presented with bleeding, presenting symptom served as an important preliminary prognostic indicator in this series. The clinical spectrum of the disease and the distribution of patients according to age in the present study is similar to that reported by Western studies and some Indian studies.^{7,8,9,10}

As nuclear grading is included in the revised FIGO recommendation, a precise definition of nuclear atypia will be of great relevance. The nuclei of malignant cells are characteristically larger, less regular in outline, more densely staining and have larger and more numerous nucleoli, than those in benign or normal cells. These characteristics are quantifiable and have shown to be prognostically relevant in some morphometric studies.^{11,12} Connelly et al proposed a nuclear grading system and found that it predicted the subsequent clinical course better than the histological grade¹². They found more neoplasms with histological grades at higher levels than the nuclear grade. However, Geissenger et al observed that nuclear grade was greater than histological grade⁴. In the present study, observations similar to that of Connelly et al were made; however, this study had in addition morphometry which overcomes the limitations of subjective assessment. By selecting the area of section containing the largest and most atypical nuclei, nuclear area was found to show significant correlation with tumour grade. Further, parameters like the nuclear

perimeter and nuclear shape will help additionally, rather than isolated assessment of nuclear area for correct morphometric grading.

The mitotic index and mitotic count are well established procedures in tumour grading.¹³ Though they seem simple, there are major limitations. The recognition of mitotic figures is subject to observer error and the histologically visible event of mitosis occupies only a short period in the cell cycle, so a large number of nuclei have to be counted to obtain a statistically reliable estimation of the proportion of proliferating cells. Any delay in fixation results in underestimation of the number of mitosis.¹⁴ In the present study which was a retrospective analysis, the fixation details were not known. Hence the presence of mitotic figures was observed but not quantified.

Among the other morphological features, depth of myometrial invasion correlated well with the prognosis, probably because the deep myometrial invasion was observed only in poorly differentiated carcinomas. Necrosis and lymphoplasmocytic response, which were observed in well and moderately differentiated adenocarcinoma, also showed correlation with better prognosis.

The presence of endometrial hyperplasia may demonstrate a more favourable prognosis. In a study which compared patients with carcinoma with and without hyperplasia, those with hyperplasia were better differentiated, lacked deep myometrial invasion, cervical involvement and lymphovascular space invasion.¹⁵ In the present series, concomitant hyperplasia was observed in the non-neoplastic endometrium in 5 of the patients. Though the number is too small for statistical evaluation, it is noteworthy that tumour in these patients was of Grade 1 or well differentiated.

There is a danger of well differentiated carcinoma being incorrectly diagnosed as hyperplasia as carcinomas are rare in those below 40 years of age. In such cases, nuclear morphometry which can be easily done by eyepiece micrometry can be applied to aid in diagnosis and further evaluation.

In summary, the diagnosis and grading of endometrial carcinoma can be based on few simple procedures in addition to light microscopy, which when carefully performed will permit adequate evaluation of the tumour. This will have impact on the treatment which will lead ultimately to better cancer control.

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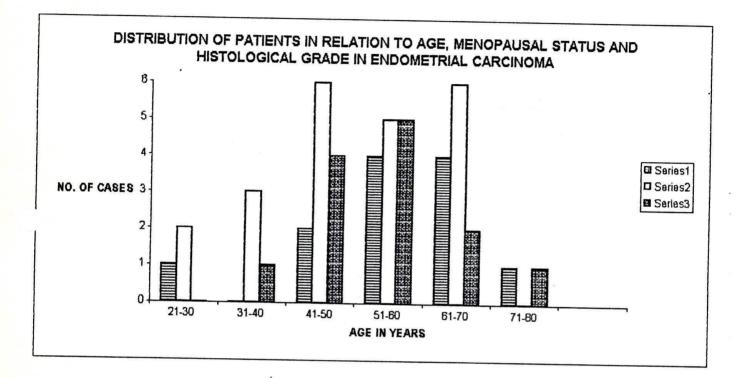
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CORRELATION BETWEEN HISTOLOGICAL GRADE AND NUCLEAR FEATURES IN ENDOMETRIAL CARCINOMA

GRADE	NUCLEAR AREA (μm²) MEAN ± S.D	NUCLEAR PERIMETER (µm) MEAN ± S.D
WELL DIFFERENTIATED	51.2 ± 2.41	36.11 ± 0.97
MODERATELY DIFFERENTIATED	42.54 ± 3.69	33.47 ± 1.83
POORLY DIFFERENTIATED	37.12 ± 3.91	30.72 ± 1.92
ANOVA	F VALUE = 28.79 p VALUE ≤ 0.001	F VALUE =5.53 p VALUE ≤ 0.01



Sheet1

STEPS IN CARRYING OUT ANY RESEARCH

- Describe and define study problem. 1.
 - Background, arguments for relevance, aspects related to study subject.
- Formulate / define study question. 2.
 - Start with broad definition, try to specify / narrow down the question(s) to be answered in a series of subsequent steps.

Mari Kallath

- Make sure that all the terms / concepts occurring in the study question are stated in concrete / measurable term ('operationalization'). You should be able to explain all the terms.
- Study the relevant literature. Make a review / overview / meta-analysis / research synthesis. 3. What is already known about the problem to be studied, from previous investigations (frequency of occurrence, related phenomena, potential confounders, etc.)? What are the 'white spots'? What were the methodological weaknesses of previous research efforts?
- Reformulate the study question (based on the conclusions from the literature study). 4. Distinguish several subquestions. Can the be answered all at once, using the same study, Formulate research hypotheses (if possible; hypothesis testing research vs. exploative research).
- Consider the nature of the study question. Does it require a descriptive or an analytic (cause-5. effect) type of approach/research. This distinction refers to the study objectives, so on the intended applications of the study results. Does one just want to describe the frequency of occurrence of one or more relevant phenomena (e.g., a particular disease, symptoms, immune status, a risk factor), including differences in their distribution between subpopulations based on time-, place-, and personcharacteristics. Or does one intend to end up with conclusions in terms of (causal) relationships between phenomena/variables measured.
- If the study problem is health/disease-related, consider which phase of the natural cause of 6. disease is involved (preclinical / clinical; asymptomatic / symptomatic; etiology / diagnosis / prognosis; prevention (primary/secondary / treatment (tertiary), etc.). Is there yes/no an intervention among the phenomena to be studied?
- Design options. 7.
- 7.1. Provisional choice of study design:
 - Level of aggregation of measurements / observations:
 - · Group level : ecological study, correlation study (geographical correlation study, time series analysis)
 - · Individual level : other types of study
 - Level of comparison:
 - No : e.g., case study, case series
 - Yes : other types of individual studies
 - Timing of study:
 - cross-sectional study, survey study One measurement time-point 1
 - Several measurement time-points : longitudinal study:
 - · prospective: follow-up study, concurrent cohort study (no random allocation), experimental intervention study (random allocation) · retrospective study: case-control study
 - For intervention studies in the social sciences the classification scheme introduced by

Analytic studies - internal validity imp Description studies - Get. validity imp.

Cook & Campbell is often used: three main groups of disigns are distinguished: preexperimental, guasi-experimental en true experimental designs, each containing several designs (notation system: O = observation, X = intervention, E = experimental group, C = control group; types of bias: history, maturation, selection, attrition, statistical regression. attrition, instrumentation).

2

- ' General considerations in choosing a design:

Epidemislogy

- · Does the underlying study question refer to a descriptive problem (occurrence, distribution of relevant phenomena in the population; differences between subpopulations; associations between phenomena / characteristics) or to an analytic problem (1, determinants / risk factors / etiologic factors. 2. effects of planned interventions). When dealing with descriptive problems it is very important to work with a representative sample (external validity !), for analytic problems one may choose a non-representative way of sampling that guarantees a high level of internal validity.
- To attain a high level of internal validity: Prevent / control for: selection bias, information bias, confounding:
 - Maximal contrast in status of central exposure / intervention factor
 - Minimal contrast in status of other disease / outcome determinants: restriction, randomisation, prestratification, matching, stratified analysis, etc. No measurement errors (systematic, random): measurement protocol, blinding, etc.
- · Pursuit of a high degree of efficiency: statistical (precision), economical (money, personnel, time needed)
- 7.2. Development of study design:
 - 1. Choice of study population (who should be assessed, treated, etc. ?):
 - Composition: inclusion- and exclusion criteria (eligibility criteria, admissibility criteria)
 - Size of study population
 - Selection procedure:
 - Source population?
 - Sampling frame available?
 - · Sampling procedure
 - Methods: probability samples (known and often equal chance for each member of the population to be sampled; e.g., simple random sampling, stratified sampling, cluster sampling) versus non-probability samples (e.g. snowball sampling, haphzard sampling, etc.)
 - Method of recruitment
 - 2. Choice of measures (what should be measured?)
 - Phenomena to be measured (conceptual level)
 - Measurement tools: available or to be developed? -
 - Attention for:

4

- Validity
- (face-, content-, construct-, criterion- (concurrent, predictive): Se, Sp, LR, PV+, PV-, etc.)
- Reproducibility / Precision
- (inter- and intra-rater reliability, test-retest reliability,
- internal homogeneity / consistency: Kappa, r, etc.) Sensitivity to change / responsiveness (in case of effect measure)
- Practical applicability
- Methods of data collection needed (records, registers, interviews, guestionnaires, observation, physical examination, laboratory analysis)
- Other aspects of measurement procedures (e.g.: 'blinding' of observers / raters)
- 3. Timing of measurements (when, how often, what sequence)
- 4. Way of allocation of study subjects to exposure factors / intervention factors:
- Random allocation vs. self-selection
- 5. Methods of data handling:
 - Storage
 - Statistical analysis: protocol

1

-> what happens in protocol deriction

- · Univariate analysis: frequency distribution of each variable (mean + s.d., median + Inspecting and handling missing values, outliers, illegal values: data control
 Bivariate analysis: cross tables, stratified analysis

- · Multivariate analysis: e.g. multiple regression, logistic regression, discriminant analysis.
- 7.3. Write down study design in protocol, including time schedule. Discuss with collegues, funding agents, research committees, etc.
- Preparation / organization of the study. 8.
 - Appointments with 'the field'
 - Development of questionnaires, forms, etc.
 - Development of measurement tools
 - Attention for legal requirements
 - Fainancial aspects
 - Housing + other facilities
 - Etc.
- Conduct of the study. 9.
 - Collecting data, control of data, additional information, enter data in computer
 - Etc.
- 10. Data analyis.
- 11. Reporting:
 - Reports
 - Journal articals:

Structure: Introduction: motivation, study question

Background information

Study design (Material and methods): population, measurements, analysis Discussion

4

witten.

Conclusions + Recommendations

STEPS IN AN EVALUATIVE INTERVENTION STUDY

- Define the intended intervention effect:
 - Nature of outcome measure:
 - Relevance

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- Measurability
- Conceptual (e.g.: 'functional disability') --> Operational, concrete (e.g.: a specific disability questionnaire or rating scale (e.g.: 'Roland Disability Scale')

- Measurement tool: Existing one <----> newly developed one

Clinimetric qualities:

- validity (face-, content-. construct-, criterion- (concurrent, predictive): Se, Sp, PV+, PV-, LR, etc.
- reproducibility / precision (inter- and intra-rater reproducibility, test-retest reliability, internal consistency / homogeneity): Kappa, r, etc.
- · sensitivity of change / responsiveness
- practical applicability (costs, time needed, hazards, etc).
- Size + direction of effect
- Time reference: How much time will it take before a possible effect will be visible, will fade away? When and how often should outcome status be measured?
- 2. Define intervention(s):
 - a. Experimental intervention:
 - New programma <--> Existing programme Or; new elements added to existing programma
 - Broadly defined, complex, multi-focal, national level <---> specific, local level
 - b. Reference / comparison intervention:
 - Usual care
 - Placebo intervention
 - No intervention at all
- 3. Define base population + study population
 - Composition
 - Inclusion- / Exclusion criteria (Eligibility criteria, Admissibility criteria)
 - Restriction (homogeneous population), matching, etc. in order to prevent bias (confounding, selection bias) = to improve comparability / validity, to improve efficiency.
 - Sampling procedure
 - · Size (N)
 - Depends of size of effect / amount of change in reference group, extra effect / amount of change that one wants to detect in the experimental group, accepted chances of type I- and type II-errors

-

- Anticipate of non-response (%), attrition during follow up.
- Procedure of acquisition (source (e.g.: advertisement, hospital, screening programme); informed consent procedure (--> non-response)
- 4. Allocate eligible and available candidates to intervention alternatives:
 - Self-selection
 - Randomization (+ pre-stratification)
- Measure base-line values (outcome variables, potential confounders / effect modifiers)

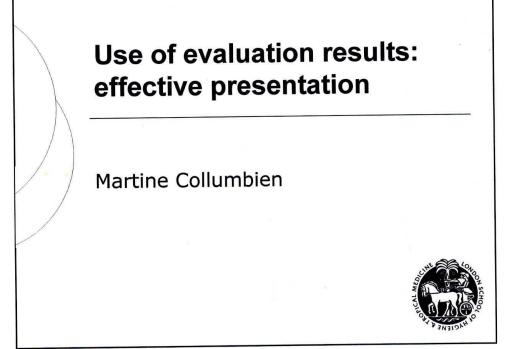
Implement intervention programme:

- Standardized protocol <---> Individualized intervention
- Control group: Placebo intervention
- 7. Control + measure compliance + attrition:
 - Contamination of subgroups: result of randomization procedure will be overruled

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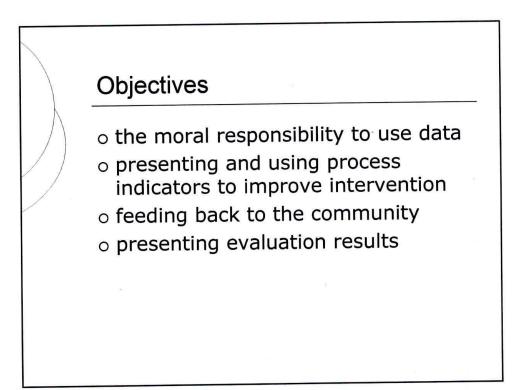
- Stimulate + measure (questionnaires, biological markers)
- Placebo-intervention will improve the level of compliance
- Attrition: selective or non-selective.
- Conduct follow-up measurements (how often? when?)
 - 'Blinded' measurement
- 9. Be alert for (unwanted, adverse) side-effects (observations, questionnaires, register)

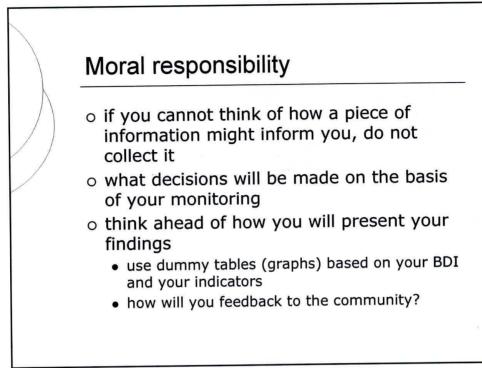
10. Analysis.

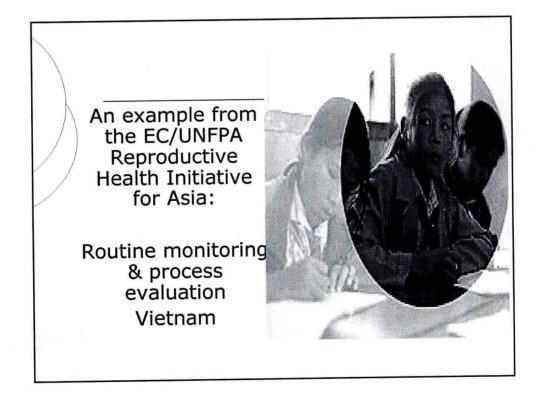


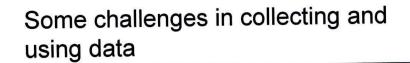
Day

RES-1.

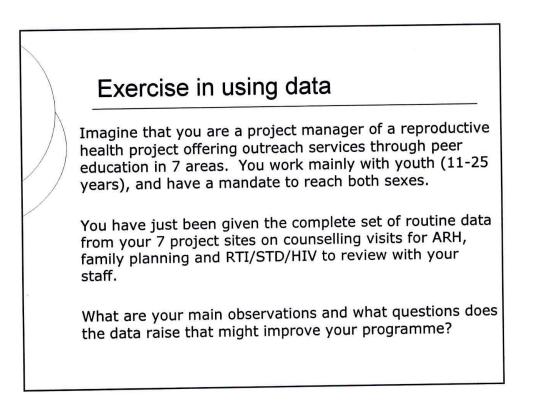




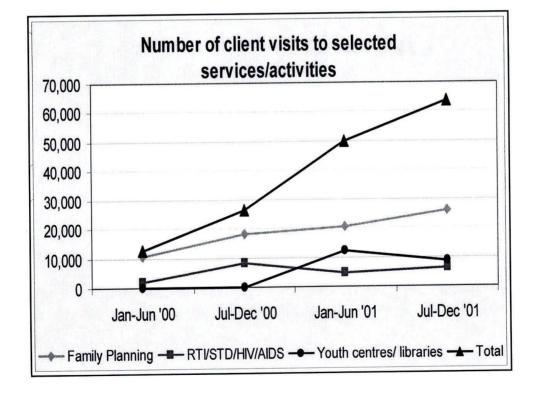


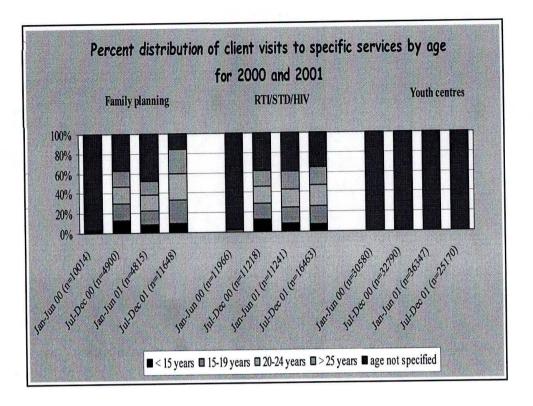


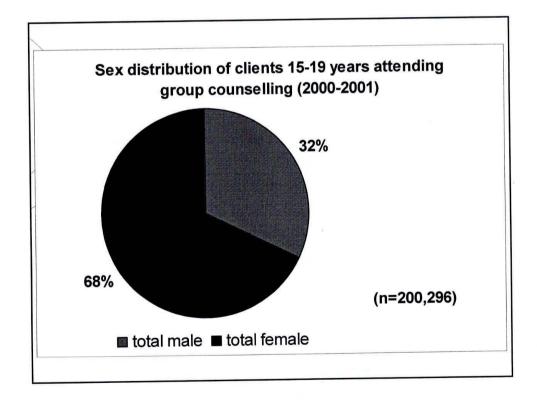
- May be common sense to collect data by sex, but many NGOs did not do it when we started.
- Even when they did, data is often aggregated, and few analyse it by sex.
- Similar with age
- Data often not integrated into project management, or fed back to project staff, so little time for reflection and adjustment.

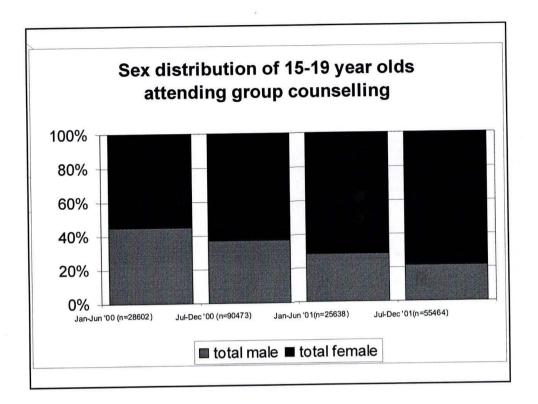


		Reporting period								
Y	Client visits to specific services	Jan-Jun '00	Jul-Dec '00	Jan-Jun '01	Jul-Dec '01					
Fam	nily Planning	10,490	18,273	20,604	25,908					
RTI S	/STD/HIV/AID	2,135	8,149	4,879	6,234					
	th centres/ braries	n/a	n/a	12,043	8,808					
Tot	al	12,625	26,422	50,108	63,701					

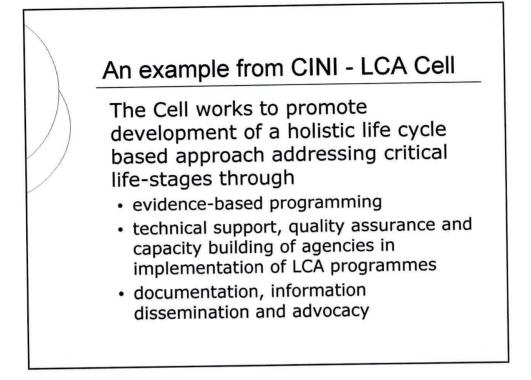


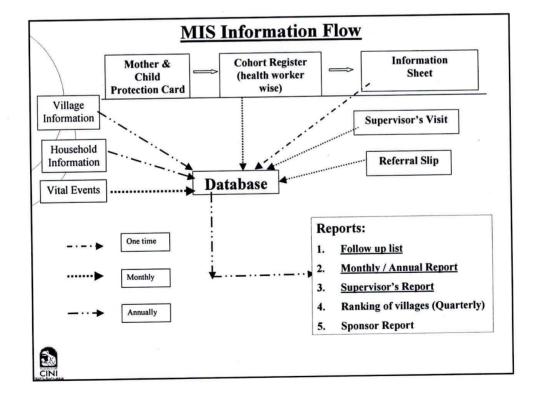


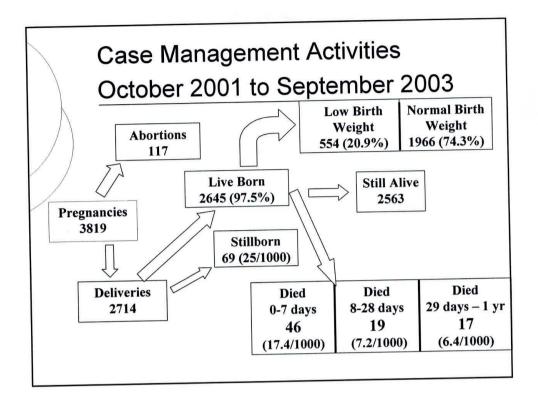


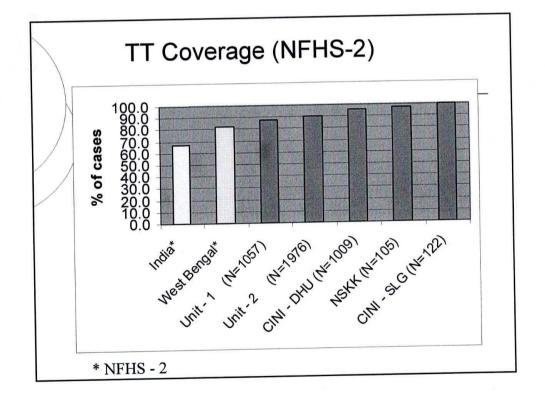


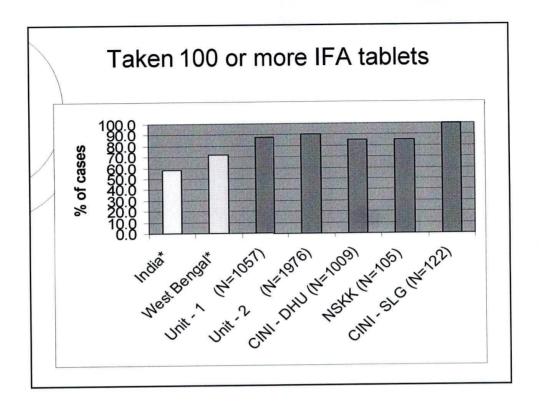
Observations from routine data on counselling
More females than males
More males seen for RTIs/HIV than for family planning
Some evidence that contact with males declines over time
Are we reaching our intended target group? What is the age profile?



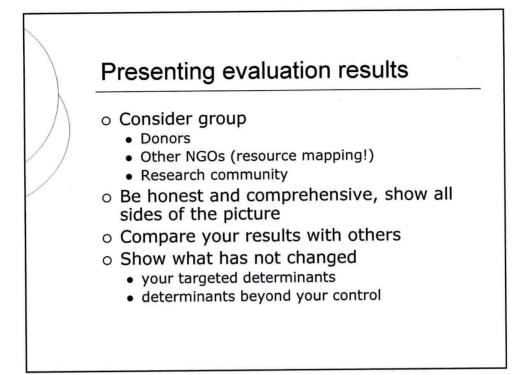






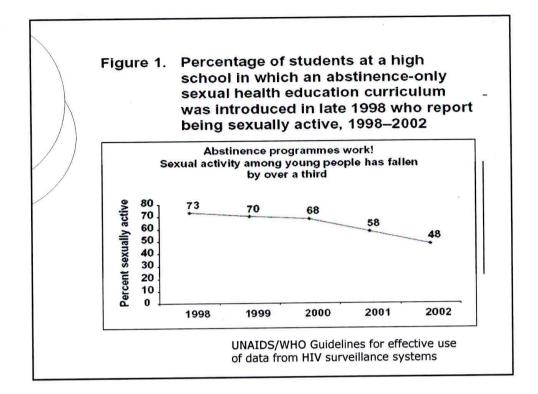


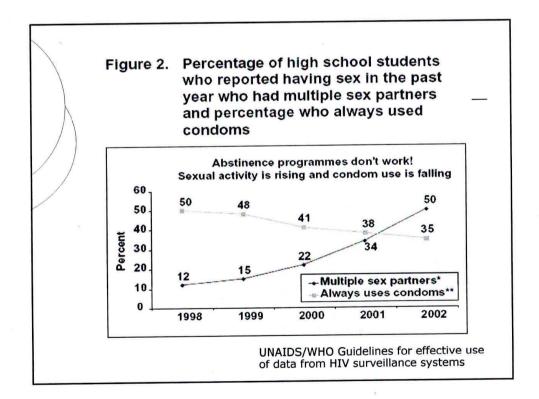
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Name of Village	N in yr 1	N in yr 2		t_16 eks	T	.T	IF	A		ttd. eliv	LE	3W		onat leath	# gra abo	ide		forma ice
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Chak Enayatnagar	56	116	A	A	A	A	A	A	В	A	A	A	В	В	4	5	67	83.3
Mirpur	37	57	A	A	A	A	A	A	B	В	A	В	A	B	5	3	83	50.0
Enayatnagar	44	69	A	A	A	A	В	В	В	В	A	A	В	В	3	3	50	50.0
Udayrampur	23	35	В	A	A	A	A	В	В	A	В	В	В	В	2	3	33	50.
Sukhdevpur	7	16	В	A	A	В	A	B	A	A	A	B	В	A	4	3	67	50.
Fakirpara		10		A		A		В		B		В		A		3		50.
Ramkrishnapur	22	68	В	A	A	В	В	A	A	A	В	A	A	A	3	5	50	83.
Chandi (S)	40	73	A	A	A	A	A	A	В	A	B	A	A	A	4	6	67	100.
Chandi (N)	40	95	В	A	В	A	A	B	A	A	A	A	В	В	3	4	50	66.
Kriparampur	31	55	A	A	В	В	В	A	A	A	A	В	A	В	4	8	271111711	50.
5	300	594													4	3	59	63.

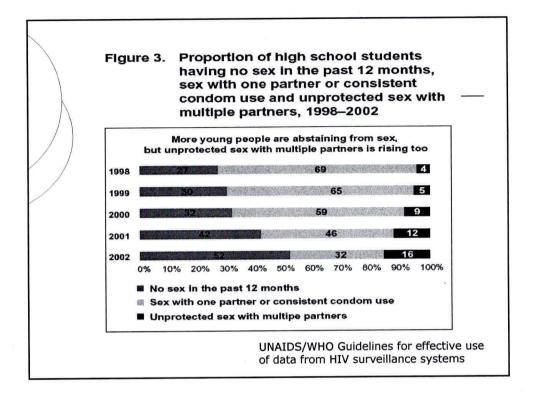


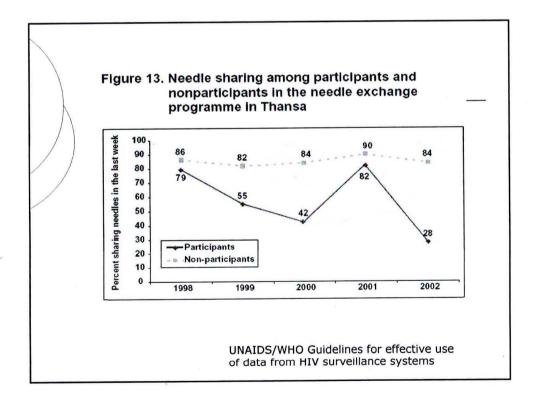
F	ull or sele	ctive data	presentation
Table 2. Re Year	Had sex in the past year	haviour among high school stud Had multiple partners in the	Aiways used condoms
	(% of all)	past year (% of all)	(% of those with multiple partners
1998	73	9	50
1999	70	11	48
2000	68	15	41
2001	58	20	38
2002	48	24	35

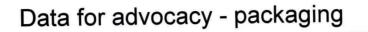
UNAIDS/WHO Guidelines for effective use of data from HIV surveillance systems







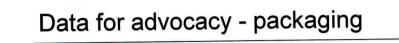




- Define your goals and audience
- Find out what influences their thinking, what their biases may and use the data
- Use the right language to communicate ...

Same information, different language

- The HIV incidence in the 15- to 19-yearold cohort is high, and the prevalence among 19-year-old women is 33%
- New HIV infections are common in the late teens; a third of 19-year-old girls are already infected with the virus
- Hundreds of teenagers get infected with HIV every week. If there are 30 girls in your daughter's class, about 10 of them will have HIV by the time they



0 ...

- o get the length right
- \circ choose the messenger
- o strategic timing
 - avoid election time or festivals
 - around World AIDS day, Women's day, Global conferences