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Evaluating an alternative cost effective protocol to screen and detect oral pre-cancerous and cancerous lesions

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Abstract
Background: Oral cancer is one the leading causes of cancer mortality nowadays, and early screening of pre-cancerous lesions is a high priority for reducing both morbidity and mortality associated with oral cancers.
Methods: The study group composed of subjects from West Bengal to evaluate the protocol for the detection of precancerous and cancerous lesions of the oral mucosa.
Results: Toluidine blue, Cytology with Leishman Giemsa stain and Histopathology methods from biopsy was combined to develop a screening protocol named Mukherjee Bose protocol. The sensitivity of this tests holds nearly at 96.2% and specificity at 77.7% in this study. The average cost to screen patients by this method was reported very cheap. By combining this three very sensitive techniques in the study were considered to make the protocol reaching a very high-level of sensitivity and efficiency.
Conclusions: The purpose of this article is to initially implement and evaluate a rapid and cost effective protocol by using present diagnostic aids used in daily practice for screening pre-cancerous and cancerous lesions.

Keywords: Oral pre-cancer, screening, prevention, diagnostic tools

1. Introduction
Oral cancer in today's world is now a menacing health problem and now accepted as Indian disease, despite advances in oral cancer therapy, it remains a disease of later diagnosis. Oral cancer among various types of cancer is an imminent problem nowadays in the Indian population, it is the sixth most cancer reported worldwide in population and basically found in both sexes. 200,000 new cases reported annually worldwide, two-third of which occur in developing countries including India with high mortality rate. Oral and oropharyngeal cancers account for 4% of cancers in developed countries and up to 40% in developing countries [1]. Prevalence order of the oral cancer was highest in developing countries around Melanasia, South-Central Asia, Central and Eastern Europe followed by Africa, Central America and Eastern Asia for both sexes as habits such as smoking tobacco, alcohol use, consuming smokeless tobacco and betel quid contribute as the major risk factor in this regions [2]. As the Asian countries are developing and still relatively poor to its western counterparts, the presence of advanced healthcare systems remain limited in these regions till date and access to the systems are limited too due to lack of awareness and money. In general, oral cancers have a tendency to be detected at a late stage and incidence worldwide remains around 500,000 new cases or 3% of malignancies every year [1]. As the highest rate of incidence is in developing countries and about 45%-55% of oral cancer patients are Indians, India was chosen for conducting the study. Figure 1 describes the epidemiology risk factor associated with oral cancer. Being a developing country with limited infrastructure, an abundance of adequately trained English speaking professionals combined with a low economy in comparison to all developed and many developing countries, this place provides an abundance of cases. Also amongst the country it was found that 72% of the cases were found in Eastern and North East Zone, so the screening was initiated in this region due to plenty available cases encompassing the criteria of being economically poor, lacking awareness, having unhealthy lifestyle, diet, habits and having limited access to good infrastructure in healthcare [3].

Oral cancer completely can be defined as a malignant neoplasm in the oral cavity, most frequently occurring oral cancer in India occur with its premalignant conditions like Oral Squamous Cell Carcinoma (OSCC), Oral Submucous Fibrosis (OSF), Erythroplakia, leukoplakia, lichen planus. Oral cancer can be defined as uncontrolled cell growth along with lesions in the oral cavity basically it starts from any tissue of the mouth and invades any other neighboring areas in the mouth. The most common presenting features are prolonged ulceration which does not heal, referred pain, to the ear, difficulty with speaking and opening the mouth or chewing and ultimately it leads to death [4]. Mostly people are diagnosed with premalignant lesions like leukoplakia and erythroplakia white and red patches covering the oral cavity. These lesions are further sub divided according to their types, homogeneous (flat, thin), non-homogenous (speckled). According to literature surveys leukoplakia and oral submucous fibrosis is the most common occurring lesions in patients with frequent duration of chewing betel quid, tobacco and it contains arecadine, arecoline, tanin, a harmful substrate when it comes in contact with the oral cavity it leads to activation of T cells, Macrophages, IL6, TNF-α and other metabolites under its influence fibroblast differentiate and leads to accumulation and formation of collagen in oral mucosa [5, 6]. The purpose of this article is to initially implement and evaluate a rapid and cost effective protocol outlined by a systematic approach of already present basic diagnostic aids such as Toluidine blue, Cytopathology with Leishman Giemsa stain and histopathology to Screen Pre-cancerous and Cancerous lesions so that with further studies and research a single screening technique accepted globally can be made beneficial to help large population areas.

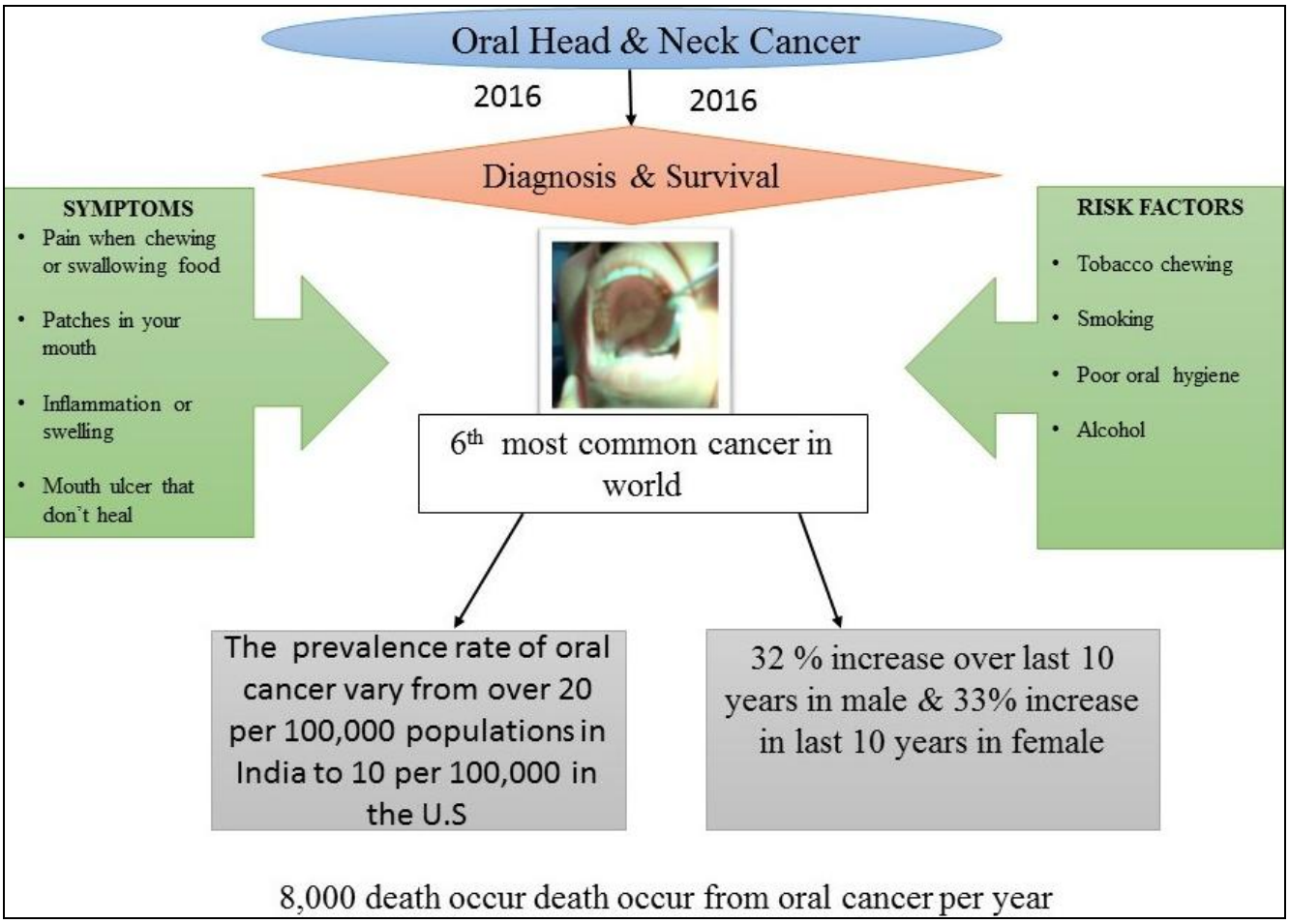

Fig 1: Describes the epidemiology risk factor associated with oral cancer

The protocol named Mukherjee Bose Protocol was established by Dr. Gopeswar Mukherjee and Dr. Surajit Bose in an attempt to provide a single universal systematic rapid approach to cancer screening as the only effective treatment is as early detection as possible, followed by surgical intervention to completely free affected areas in adjunct with chemo and radiation therapy and palliative care to help as many people as possible in a low-income strata with low awareness amongst developing countries who don't spend substantially on healthcare and relieve a small fraction of the ever-increasing burden on cash-strapped individuals and also relieve burden of increasing expenses of GDP amongst developed countries by being cost effective. Figure 2: Depicts the Mukherjee Bose protocol for oral cancer screening program [3].

Criteria generally determined by Dr. Mukherjee and Dr. Bose to uniformly select cases and make the protocol universally acceptable:

- Cases between 18-65 years, all male and female subjects to be included in this study.
- Cases having habits of taking tobacco and alcohol consumption.
- Cases coming from poor economic status/slum areas where some nutritional deficiency may be present.
- Cases that are exposed to chemical industry or radiation.
- Cases having a familial history or previously diagnosed malignancy or premalignant lesion.

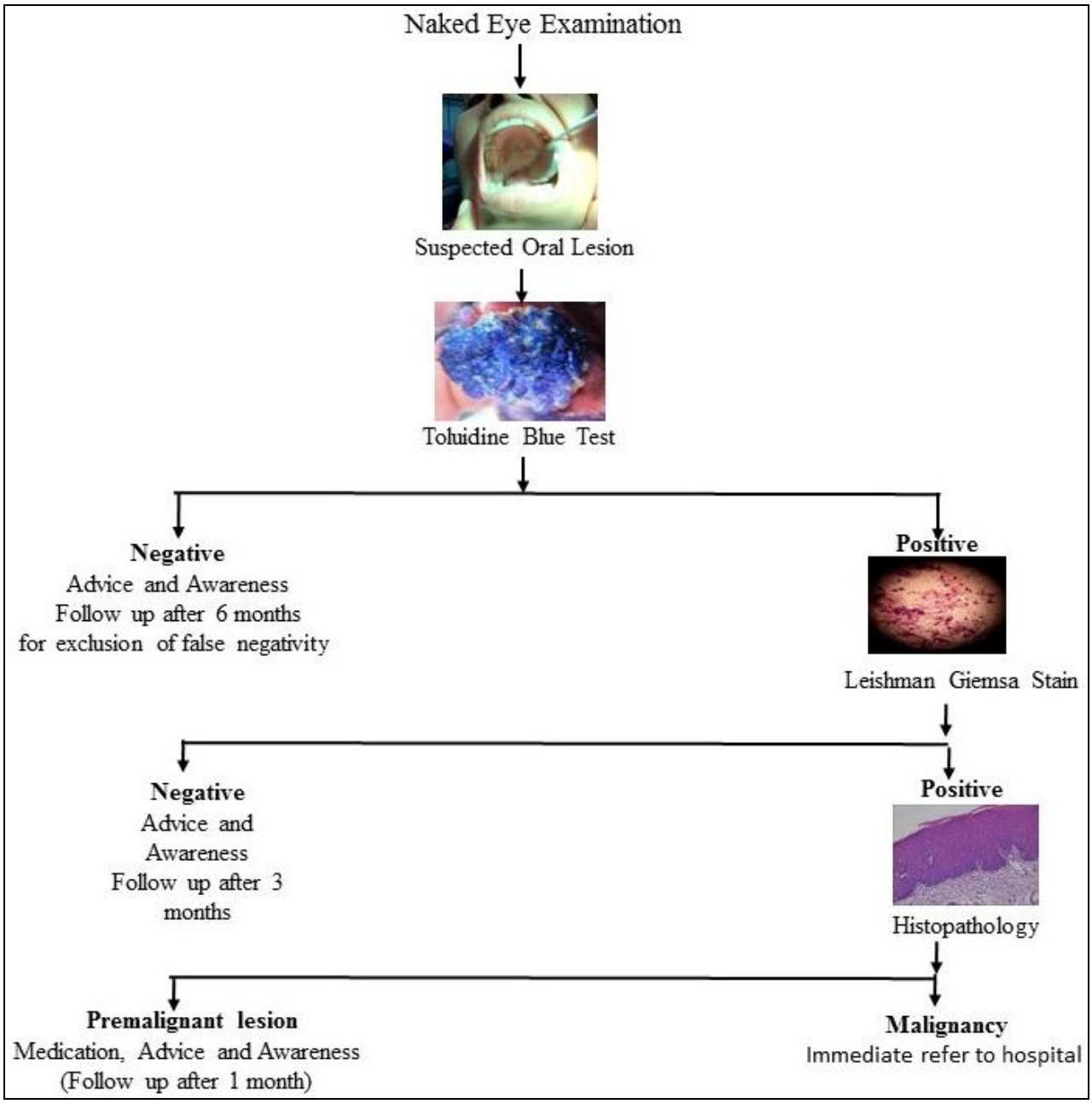

Fig 2: Depicts the Mukherjee Bose protocol for oral cancer screening program

2. Materials and Methods

2.1 Study population and sampling

The Mukherjee Bose Protocol established by Dr. G Mukherjee and Dr. S Bose in the city of Kolkata, the easterly Indian State of West Bengal was chosen to be the alternative protocol as it met all the criteria for a favorable region needed according to the statistics of cancer incidence worldwide. Kolkata, West Bengal was both a favorable place as a city to enjoy benefits of studying large and varied populations, have the infrastructure to support the study and be in one of the most populous caries incidence zones. Yet the city provided with limited access to advanced and expensive healthcare along with the low economy, low awareness and display continuous habits of all major risk factors. The study using the protocol for the cancer screening program was conducted in four centers along with ethical clearance from Bharat Sevasram Hospital, Joka; Smile and Profile Dental Clinic Rajabazar; Aeolian Dental Clinic, Hatibagan and Barasat Cancer Hospital, Barasat. All concerned institutional authorities were duly informed and patient consents were taken before the procedure. The study was conducted for 6 months from November 2016 to April 2017 amongst 2575 patients turning up in the OPD for oral and dental complaints in all the four centers. The centers all varied from a Government Hospital with minimal working infrastructure suited to address cancer needs, a Not for Profit General Hospital lacking the advanced infrastructure needed to cater to treat cancer and two general private dental clinics with only chair settings for general dental practice thus lacking the necessary infrastructure needed there as well.

Fig 3: Showing the Map of West Bengal and its position within India. The red arrows indicate the districts of West Bengal, the state of Jharkhand (to the extreme left) and the neighboring country of Bangladesh (to extreme right) from which the 50 cases used for study hailed. This gives an idea of the geographic location of the incidence of oral cancers occurring. Photo courtesy: maphill.com.

A total of 50 (19 male, 31 female)suspected patients; within the age group of 18-65 years(4 female and 2 males above 65 years) by naked eye examination were screened and sent to the first stage of toluidine blue staining test [7]. The toluidine blue is a basic thiazine dye with high affinity for acidic tissue components [8]. All suspected lesions were examined by Pathologist and dentists with a considerable amount of knowledge in head and neck cancer lesions.

2.2 Sample collection methodology

Patients were made to rinse the oral cavity with water for half a minute to remove debris and rinsed with 1% acetic acid for 20 sec. Toluidine blue (1% W/W) was applied as an oral rinse for 20 sec and then 1% acetic acid was again used for 20 sec to eliminate mechanically retained stain. Lesions showing dark blue staining were considered to be positive for premalignant or malignant tissue, while those with light staining or totally not colored were negative. This was repeated after 6 months to rule out false negativity [9].

Of the 31 cases found to be positive, patients were subjected to scrap cytology technique, where upper layer of the lesion was scraped with a tongue blade and transferred to a microscope viewing slide and cells smeared with Leishman Giemsa stain after which it was fixed immediately in wet condition by dipping into a container filled with fixative and viewed under microscope. An increased nuclear-cytoplasmic ratio showing hyper chromatic nuclei with chromatic clumping in cytoplasm and irregular, angulated nuclear borders tested positive for possible inflammatory, candidiasis, premalignant and malignant lesions. Cells appearing normal turned out to be negative [10]. This scrape cytology would be repeated after 3 months for patients tested positive with toluidine blue but negative in cytopathology with Leishman Giemsa to rule out false negativity.

Lastly, those tested positive with cytopathology were sent for biopsy to provide absolute confirmation in addition to the information already present with cytopathology reports. Both intra oral, 9 incisional and 11 excisional out of 20 biopsies were performed by an oral pathologist under local anesthesia and using surgical blade following standard chair side minor

oral surgery procedures. The tissue obtained was sent to the pathology lab, sectioned and fixed onto slides to be observed under a microscope for findings. Pathologic findings included several of the severe architectural changes (severe dysplasia) in the epithelium characterized by the presence of irregular epithelial stratification, loss of polarity of basal cells, presence of drop shaped rete ridges, increased mitotic figures not limited to basal or parabasal cells, premature keratinisation in single cells and keratin pearls within rete ridges as guidelines set by WHO for malignant lesions. WHO's criteria for cytologic changes in the epithelium are abnormal variation in nuclear size, shape, cell size, cell shape, increased nuclear cytoplasmic ratio, increased nuclear size, atypical mitotic figures, increased number and size of nucleoli and hyperchromasia; several of which were severely present for malignant lesions. A low grade of mild to moderate amount of dysplastic features was seen for premalignant lesions and thus differentiated accordingly [11]. The lesions tested negative and considered premalignant are to be checked back after 1 month for possible signs leading to malignancy and to rule out false negativity. The pre malignant lesions are finally advised medication and kept under close monitoring along with counseling for awareness. The positive cases were however referred immediately to the hospital for surgical intervention.

3. Results

Clinical characteristics of patients and controls are listed in Table 1. Oral cancer incidence is more than double in females [31%] than males [38%].

Table 1: Clinical details of the patients at the time of enrolment

Parameters	No. of patients [n=30]	Control Group [n=20]
Age		
Range	18- 85 years	18- 85 years
Mean	50.6 ± 15.8	48.8 ± 11.2
Male	14(46%)	5(25%)
Female	16(53%)	15 (75%)
Habitual betel quid chewer		
YES	19 (63%)	-
NO	-	31 (95%)
Site of involvement		
Whole	3(10%)	N.A
Palate	2(6%)	N.A
Tongue	6 (20%)	N.A
buccal mucosa	14(46%)	N.A
Gingival Buccal Sulcus	3(10%)	N.A
Lip	2 (6%)	N.A
Screened by Mukherjee/Bose protocol		
Positive cases	30	N.A
Negative cases	N.A	20
Symptoms		
Pain in opening mouth & increased sensitivity	18(60%)	N.A
Red spots & White patches	8(26%)	N.A
Ulceration	4(13%)	N.A

Of the 50 lesions suspected by the naked eye and stained with toluidine blue, 31 lesions stained to be Positive. Rest 19 lesions did take up a mild stain or no stain and were therefore considered Negative. Patients were provided awareness and made to follow up after 6 months to rule out false negativity.

The 31 positive cases subjected to cytopathology with Leishman Giemsa stain showed 20 cases to be Positive and 11 cases to be negative. The 11 negative cases showed no altered nuclear cytoplasmic ratio even though many were considered pre malignant lesions by nature while clinically diagnosing them. Patients were provided awareness, made to follow up after 3 months to check for transformation and increased ratios as well as rule out for false negativity.

Out of 20 cases positive in the second step of the protocol, 6 tested positive for malignancy while 14 tested negative revealing pre malignant lesions. The malignant lesions were sent for hospitalization and surgery while pre malignant lesions were given medications, provided awareness and kept under close monitoring. The negative cases were made to follow up after 1 month to rule out false negativity.

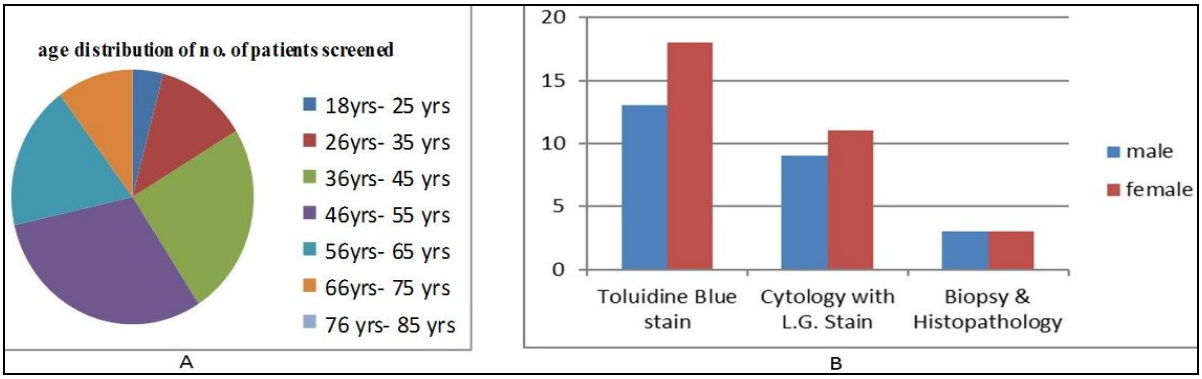

Fig 4(a): Pie diagram showing age distribution of the suspected 50 patients accounted for screening by the protocol (b) Bar chart showing no. Of male and female patients screened in each step of the protocol's different stains.

Table 2: Showing the number of cases screened sequentially using various stains in different steps as outlined by the protocol.

Methodology entity	Total no. of Cases	No. of Positive Cases	No. of Negative Cases
Suspected Oral Lesions	50	-	-
Toluidine blue staining	50	31	19
Cytology with Leishman Giemsa stain	31	20	11
Biopsy and Histopathology	20	6	14

4. Discussion and Conclusion

In this article, we have tried to provide a working data of the protocol. Of the 50 patients assessed, 6 patients were found out to be Positive for Malignancy getting us an efficiency of 12% in only 6 months. As per the assessment cited as an example of the protocol for screening of 10,000 people and able to save 250 of them in 5 years the target can be reduced to 50 people being saved out of 2000 people screened per year. So, as per the results attained by the study of 6 people could be saved out of 50 people screened in 6 months than 12 people could be saved for 100 people screened in a year. Multiplying 20 times, a population of 2000 screened could possibly save 240(4.8 times more) people against the number 50 in a year and 1200(against 250) out of the target population of 10000 hypothetically claimed to save in 5 years by the protocol in working condition going by the theory of multiplication. Though actual statistical analysis needs to be drafted by further screening large number of the population actually and repeating the steps over to rule out false negativities. From the results obtained we need to evaluate the efficiency and specificity of the protocol so that it can be further developed and used to screen large population numbers in the future.

Toluidine blue, Cytology with Leishman Giemsa stain and Histopathology from biopsy was combined to develop a protocol in the intent of all the more enhancing the specificity of the various aids already determined individually. The sensitivity of toluidine blue holds nearly at 96.2% and specificity at 77.7% in a study [9]. For cytology with Leishman Giemsa (LG cocktail), the difference in reports between LG cocktail and histopathology are identical and both are very advanced diagnostic aids. Moreover, LG cocktail is an easy, cost-effective one step technique and far superior in sensitivity and specificity to Pap, MGG, all other available stains, and histopathology [12]. Lastly, histopathology is considered the current gold standard for diagnosis and assessment of tissue biopsy of the suspicious lesion providing far more adequate clinical information [11]. Combining the three very sensitive techniques can make the protocol reaching a very high-level of sensitivity and efficiency.

The average cost to screen patients by this method is very cheap with prices as low as ten rupees per case when testing up to cytology is done. Only histopathology evaluation could be costlier in contrary to the other two techniques but still cheaper as whole because not all cases would be requiring an evaluation and would be screened by the other two cheaper aids before histology and also present with specific additional information [3]. Thus this method could thrive well amongst but not limited to poor people needing the test and also drive down costs in an already burdened GDP needed to spend for healthcare in developed countries. According to statistical reports, National expenditures for Cancer Care in the United States totalled nearly $125 billion in 2010 and could reach $156 billion in 2020 and according to the Cancer Research UK, additional late diagnosis of cancer by parts of the NHS costs them £15,081 to treat a patient with third or fourth stage ovarian cancer for example as against £5,328 if detected in early stages. Early diagnosis of any cancer can save the NHS nearly £210m a year and improve chances of survival for nearly 52,000 patients [2]. The protocol if implemented in the future can allow for early detection to drive costs down as seen and as it is extremely cheap it can overall benefit a lot of people across the globe.

4.1 Certain outcomes claimed that can be implemented if the protocol becomes popular

Mass awareness program for the fight against cancer and involvement of the community, Introduction of Health Education Programme (On Community level like- Club, School, N.G.O. etc), extended health education program by showing videos, drama, etc. Cancer Registry Programme especially with the emphasis on oral cancer and pre-cancerous lesions, improvement of oral health status of the community. “Save a life”, through early detection of the premalignant lesion and “Extend a Life” through early diagnosis and treatment of malignancy. An overall improvement of socio-economic status of the community [3].

As the technique is not too complicated, a small team of volunteers can be hired and vocationally trained to complete the majority of jobs along with and under the supervision of doctors in a completely mobile setting to reach out to undeveloped and backward areas to screen a large number of people.

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6. Conflict of Interest

The Authors report No Conflict of Interest.

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