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ARTICLE

Demographic Factors Associated with Health-Related Quality of Life Among Urban and Rural Tuberculosis Patients in Kenya

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ABSTRACT

Background: Tuberculosis is one of the deadliest and disabling diseases in the world today. The infection exacts its greatest toll on individuals during their most productive years. TB patients record different perceived health related qualities of life(PHRQoL) which could be attributed to certain environmental, social and physical factors. The objective of the study was to determine the demographic factors associated with the PHRQoL among urban and rural Tuberculosis patients in Kenya. Cross sectional design was adopted. The study applied the multi-stage sampling technique. Random sampling method was used to select the TB clinics that participated in the study. Simple random sampling according to probability proportionate to TB patient's population was preferred to select the study participants. Chi-square test determined association between the various demographic factors and the PHRQoL while ANOVA tests demonstrated the overall association of demographic factors and PHRQoL. Statistical Significance was evaluated at $p<0.05$. Descriptive statistics summarized and described the data. The study established that demographic factors are associated with PHRQoL ($p=0.008$). Specifically, age, levels of education, marital status and house hold size ($P<0.05$). Gender and Household head were not significantly associated with the PHRQoL ($p>0.05$). These findings will persuade the TB management policy towards developing an intervention programs directed at the social-demographic characteristics of the TB patients for improved treatment outcomes.

1. Introduction

Tuberculosis is a global health concern with serious economic and social burden to the patient and the household. Although effective anti-tuberculosis agents have been available for over thirty years, the incident rate of the disease is still increasing [1]. According to WHO, there are 9.4M incidence case worldwide. Because of the long duration of standard treatment (six months) there is a risk of treatment default by patients. Since 1993 the World Health Organization recommended the DOTS strategy through which the National governments can meet their responsibilities to treat patients and to prevent the spread of Tuberculosis [2]. At present much of the attention within tuberculosis (TB) management is spent on microbiological cure and its impact on PHRQoL from the patient's perspective is either undervalued or seldom considered. Studies demonstrate that as compared with the general population, TB patients reported deficits in their physical and mental wellbeing [3].

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The patient's charter for TB care allows the patients to evaluate the programs performance from the patient's perspective. One of the major performance indicators should be the capability of the national TB program (NTP) to address the physical and mental wellbeing of the patient. Therefore, the assessment of PHRQoL of TB patient as an additional indicator of performance will add value to the NTBP. As such, there is increased interest in the quality of life (QoL) experienced by individuals being treated for TB. According to the World Health Organization [1], quality of life (QoL) is defined as the individuals' perception of their position in life in the context of the culture and value system in which they live and in relation to their goals. On the other hand Health related quality of life (HRQoL) is a multi-dimensional concept that includes domains related to physical, mental, emotional and social functioning. It focuses on the impact health status has on quality of life. Clinicians and public health experts have used HRQoL and well-being to measure the effects of chronic illness, treatments and short and long-term disabilities [5]. Due to long duration of treatment and use of several drugs regimes, tuberculosis can lead to poor PHRQoL among patients. To determine the impact of the treatment of a disease, evaluation of the health states of patients have been undertaken [3,4].

In order to obtain information on preferences of different health states and how a health state is valued, (Anxiety, depression, mobility, self care, participation in usual activities, cognitive impairment and social participation), different techniques have been used. In recent times the multi attribute utility instruments (MAU) has been recommended in the UK for use in cost-effectiveness analysis in economic evaluation, health care workers and pharmaceutical industry [6]. The widely used EQ-5D-5L tool has five dimensions (mobility, self care, usual activities, pain/discomfort, and anxiety/depression and three levels on each dimension ("no-, some-and extreme problem"). Respondents indicate levels of health problems on a number of dimensions of health. The questionnaire responses describe the health profile of individual. These profiles capture different dimensions of health and can be translated into an index on a zero-one scale where zero denotes death and one perfect health. Although most studies where health states evaluation has been in diabetes, the instrument remains valid in other health studies applying the same approach [5,6].

A study in Iran by Zhang *et al*. [7] that assessed PHRQoL among patients with tuberculosis by comparing baseline Quality of Life between the Cases and Control according to SF-36 Questionnaire established that Age, Sex, Marriage and Education Significantly differed between the Treatment and Control groups . In a similar study done in Tehran to assess factors associated with HRQoL in tuberculosis patients referred to the National Institute of Tuberculosis and lung disease in Tehran noted that the SF-36 Sub-Scale scores were influenced by characteristics Marital status, education level, Job status and living place. There were Significant Correlations between Education Level and physical functioning, role limitations due to physical problems, bodily pain and Vitality. TB patients with primary and high school education attained better Scores than illiterate TB patients [8].

In yet another study in South Africa whose aim was to assess the overall impact of TB on the health status and on single health domains identified in the WHO definition of health, including physical, mental and social aspects established that PHRQoL improved Significantly during the period of treatment with highest improvement (95%) being observed in mental health. Young patients with higher education and who were employed had a better PHRQoL [9]. In Kenya a study to determine PHRQoL among patient suffering from Diabetes using the EQ-5D-5L observed that 46% of respondents had no problem with mobility, 92.2% self-care, 73.9% usual activities, 40.4% Pain/discomfort and 48.3% anxiety/depression respectively [10].

2. Methodology

2.1 Study Design

The Study adopted the Randomized cross-sectional assessments of TB patients. The study subjects were recruited from hospital registers in the sampled facilities.

2.2 Study Setting

The study was carried out in Kenya. Kenya is in East Africa with 47 Semi-autonomous Countries governed by elected governors. At 580,367 square kilometers, Kenya is the world's 48th largest Country by total area. With a projected population of more than 52.2, the Country is the 27th most populous in the World. Kenya's Capital and largest City is Nairobi.

2.3 Study Population

The study population was the tuberculosis Patients who attended the public health facilities for treatment in Nairobi and Murang'a Counties.

2.4 Sample Size and Sampling Technique

This study had a total sample size of 310. The study adopted the Multi-Stage Sampling technique. Kenya was purposively selected due to its large and rising TB burden in the region. Nairobi County was purposively selected

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due to its TB burden nationally. Murang'a County was selected conveniently due to its rural setting and close proximity to Nairobi County. Random Sampling was used to select the Hospitals, Health Centres and Dispensaries which participated in the study. Random Sampling Proportionate to TB Patient's population was adopted in selecting the study participants.

2.5 Data Collection

The standard questionnaire was the major instruments for data collection. The pre-test-Questionnaire collected information on the TB patient's demographic and socio-economic data such as age, gender, Patients Income, Occupation, residence, education level and employment and Household Income. The functional capacity of the patient was tested by (EQ-5D-5L) with five additional dimensions. The European Quality Visual Analogue Scale (EQ-VAS) tested perceived poor health of the patient.

3. Results

3.1 Introduction

The study sought to determine the demographic factors associated with PHRQoL among the urban and rural tuberculosis patients in Kenya. To achieve this goal public health facilities that treat tuberculosis in Nairobi and Murang'a Counties were identified for the study because of their rural and urban settings. Below is the summery of the results.

3.2 Demographic Characteristic of the Respondents

Demographic factors considered included age, gender, education, primary occupation, marital tutus, household headship and household size. Table 1 below present the demographic characteristics of the respondents.

Table 1. Demographic characteristic of the respondents

Variable	N	Minimum	Maximum	Mean	Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Statistic
Age	296	19	76	35.9696	11.92927
Gender	298	1	2	1.3893	0.4884
Education	283	1	4	2.6572	0.82461
Marital Status	298	1	4	1.7685	0.73173
Primary Occupation	286	1	6	4.014	1.65003
Household Head	288	0	2	1.3403	0.48191
Household size	241	0	8	3.5394	1.70523

The mean age for the respondents was established to be 35.9696. Most of the respondents were female (M=1.3893, Std. Deviation = 0.4884). The study also established that most of the respondents were married (M=1.7685 Std. Deviation. = 0.73173). Most of the respondents indicated that their primary occupation was informal (M=4.014, Std. Deviation = 1.65003). Further, most of the respondents interviewed were household heads (M=1.3403, Std. Deviation. = 0.48191). Of the sampled respondents, mean household size was 4 (M=3.5394).

3.3 Perceived Health Related Quality of Life

The study used a functional capacity of the patient was tested by (EQ-5D-5L) with five additional dimensions. Responses were ranked from the least challenge and to the worst challenge. Table 2 below presents the findings.

Table 2. Descriptive statistics for the quality of life themes

	N	Minimum	Maximum	Mean	Std. Deviation
Mobility	298	1.00	5.00	1.2349	.62914
Self-care	298	1.00	4.00	1.1174	.42199
Usual Activities	298	1.00	5.00	1.3624	.80583
Pain/Discomfort	298	1.00	5.00	1.3960	.68969
Anxiety/ Depression	298	1.00	5.00	1.2483	.50461
Sleep	298	1.00	3.00	1.2047	.43621
Memory/Concentration	298	1.00	3.00	1.1007	.32297
Fatigue/ Energy	298	1.00	3.00	1.2718	.46050
Seeing and hearing	298	1.00	2.00	1.0570	.23232
Contact with others	298	1.00	2.00	1.0302	.17143
Valid N (listwise)	298				

The findings of the study reveal that some of the health challenges for the PHRQoL include pain and discomfort (M=1.3960) and usual activities (M=1.3624). Least health challenge was experienced in contact with others(M=1.0302), seeing and hearing (M=1.0570), memory/concentration (M=1.1007) and self-care (M=1.1174). The responses obtained from the 10-theme PHRQoL were generally left skewed. While this was the case, about half, the total number of respondents did not indicate their overall PHRQoL as above 75%. Table 3 below presents the PHRQoL.

Table 3. Perceived Health Related Quality of Life

	Frequency	Percent	Mean	St.Dev
< 25.00	1	0.3	73.3734	17.78006
25.00 - 49.99	24	8.1		
50.00 - 74.99	121	40.7		
75.00+	151	50.8		

The mean PHRQoL for the respondents was 73.3734 with a standard deviation of 17.78006. About 50.8% of

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the respondents indicated that their PHRQoL was 75% and above. Those with less than 25% PHRQoL were 0.3% while about 8.1% indicated that their PHRQoL was 25-49.99. The PHRQoL scores were also right-skewed, with responses clustered predominantly around 70 and 90 on the 100 mm scale (Figure 1)

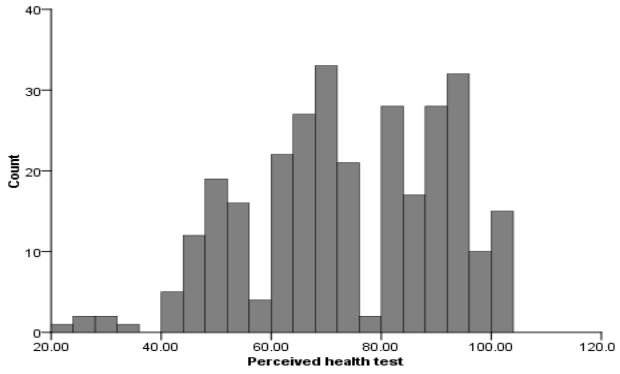

Figure 1. Perceived Health Related Quality of Life

3.4 Demographic Factors Associated with Perceived Health related Quality of Life

Demographic factors investigated for association with PHRQoL included gender, Age, education, Marital status and primary occupation. Table 3 below presents the demographic characteristics associated with PHRQoL.

The study established that demographic associated PHRQoL include age, levels of education, marital status, household size and primary occupation($p<0.05$). However, gender of the participants was not associated with the PHRQoL.

Among the different age cohorts, those aged between 35-44 had the highest mean PHRQoL at 75.0811. Those within the age cohort of 27 and below also had mean PHRQoL of 75.0233. The least mean PHRQoL was recorded among the respondents within the age groups of 28 – 34 years at M=70.3881. Respondents aged 45 years and above had mean PHRQoL of 72.4691. This was significant at p=0.04 and a chi square value of 46.311. The findings indicate that persons with

Table 3. Demographics Factors Associated with Perceived Health Related Quality of Life

Variable		Perceived Health Related Quality of Life					
		<= 25	26 - 50	51 - 75	76+	Mean	SD
Age	<= 27	1(1.2)	11(12.8)	33(38.4)	41(47.7)	75.0233	18.35466
	28 - 34	1(1.5)	16(23.9)	24(35.8)	26(38.5)	70.3881	19.00794
	35 - 44	0(0.0)	5(6.8)	34(45.9)	35(47.3)	75.0811	15.40482
	45+	1(1.5)	7(10.3)	31(45.6)	29(42.6)	72.4691	18.30253
				X^2=46.311	**p=0.04**		
Gender	Male	0(0.0)	24(13.3)	79(43.6)	78(43.1)	73.2536	16.80003
	Female	3(2.6)	15(12.9)	44(37.9)	54(46.6)	73.5603	19.28382
				X^2=46.311	**p=0.885**		
Education	No schooling	0(0.0)	5(23.8)	10(47.6)	6(28.6)	67.1429	19.53532
	Primary	0(0.0)	15(15.3)	47(48.0)	36(36.7)	70.7959	15.99804
	Secondary	3(2.5)	15(12.5)	43(35.8)	59(49.2)	74.26583	18.90905
	Tertiary	0(0.0)	4(9.3)	18(41.9)	21(48.8)	75.9767	17.33355
				X^2=139.824	**P=0.021**		
Marital Status	Single	1(0.9)	15(14.0)	48(44.9)	43(44.2)	72.6449	18.81752
	Married	1(1.6)	19(15.9)	68(41.2)	77(46.7)	74.1145	16.72227
	Divorced	1(9.1)	3(27.3)	4(36.4)	3(27.3)	65.9091	24.98181
	Separated	0(0.0)	2(14.3)	3(21.4)	9(64.3)	76.0714	15.38463
				X^2=139.824	**p=0.0331**		
Primary Occupation	Agriculture	0(0.0)	0(0.0)	5(29.4)	12(70.6)	83.7059	14.05687
	Formal Sector	1(3.8)	2(7.7)	8(30.8)	15(57.7)	77.6923	19.89526
	Informal Sector	2(1.8)	10(9.1)	44(40.0)	54(49.1)	75.3627	17.13383
	Security Agency	0(0.0)	3(25.0)	4(33.3)	5(41.7)	72.6667	22.80085
	Student	0(0.0)	5(20.0)	13(52.0)	7(28.0)	67.84	15.50183
	Unemployed	0(0.0)	16(16.8)	41(43.2)	38(40.0)	71.1684	17.44819
				X^2=180.473	**p=0.002**		
head of the household	Yes	2(1.1)	27(14.4)	75(40.1)	83(44.4)	72.8925	17.87123
	No	1(0.9)	12(10.9)	48(43.6)	49(44.5)	74.1909	17.6752
				X^2=39.61	p=0.543		
Household size	<= 2	0(0.0)	15(22.7)	25(37.9)	26(39.4)	71	19.04004
	3-4	1(0.9)	14(13.1)	49(45.8)	43(40.2)	71.4766	16.90057
	5-6	2(3.4)	5(8.6)	28(48.3)	23(39.7)	70.8776	18.29713
	7+	0(0.0)	1(11.1)	3(33.3)	5(55.6)	79.7778	15.35234
				X^2=357.286	**p=0.003**		

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ages 27 and below as well as those with middle ages (35 – 44) had the PHRQoL. Those with least PHRQoL were aged 28-34 (Youthful ages) and the elderly (45 years and above).

The study also established that female respondents indicated better PHRQoL (M=73.5603) as compared to their male counterparts (M=73.2536)

The findings indicate that respondents with Tertiary levels of education had the highest PHRQoL (M=75.9767) while those with no formal schooling had the least mean PHRQoL (M=67.1429). This was significant at p=0.021 and a chi square value of 139.824. Further, the study established that respondents who indicated that they were separated had the highest PHRQoL (M=76.0714). Those with the least PHRQoL (M=65.9091) indicated that they were divorced. Marital status was established to be associated with PHRQoL (X^2=139.824, p=0.0331). Similarly, respondents whose primary occupation was agriculture had the highest PHRQoL (M=83.7059) with over 70% of them indicating that their PHRQoL of 75% and above. Majority of the respondents within the formal sector (57.7%) also indicated their PHRQoL as 75% and above (M=77.6923). The findings also revealed that students had the least PHRQoL (M=77.6923). The study established that primary occupation was associated with the PHRQoL (X^2=180.473, p=0.002).

From the study findings, it was established that respondents who were not household heads had higher PHRQoL (M=74.1909) as compared to their counterparts who were household heads (M=72.8925). Further, it was established that respondents whose household sizes were above 7 had the highest PHRQoL (M=79.7778). On the other hand, respondents whose household sizes were 2 or less had a PHRQoL (M=71). Those whose household sizes were 5-6 members had a mean PHRQoL of 70.8776. The household size was established to be statistically associated with the PHRQoL (X^2=357.286, p=0.003).

ANOVA test on the overall association between the PHRQoL and the demographic factors were a presented in Table 4 below. A p Value of 0.008 and F=2.825 was obtained indicating a significant association between demographic factors and the PHRQoL.

Table 4. ANOVA test of the Association between Demographic factors and the Perceived Health related Quality of Life

ANOVA[a,b]					
Source	Sum of Squares	df	Mean Square	F	Sig.
Regression	5873.714	7	839.102	2.825	.008
Residual	74852.397	252	297.033		
Total	80726.111	259			
a. Dependent Variable: Perceived health test					
b. Model: (Intercept), Age, Gender, Education, Marital Status, Primary Occupation, Are you the head of the household, Household size					

4. Discussion

4.1 Perceived Health Related Quality of Life

The study established that responses on the functional capacity of the patient was tested by (EQ-5D-5L) were generally left skewed. The overall rating of quality of life was also right skewed indicating more positive rating of PHRQoL. However, half of the respondent did not rate their PHRQoL above 70%. The mean PHRQoL was less than 75% indicating general poor rating of the PHRQoL. The findings lead to an understanding that there was general positive assessment of PHRQoL and an overall percentage rating of the PHRQoL in the study area. The study established that demographic factors associated with the PHRQoL include age, marital status, level of education, household size and primary occupation.

4.2 Age and Perceived Health Related Quality of Life

Age was significantly associated with the PHRQoL. While the lower age brackets indicated higher PHRQoL, there was a decline in such rating at the next age cohort (mid youth) and improves at late youth. However, poor PHRQoL rating was registered at older age brackets. The finding leads to an understanding that at early youthful ages among TB patients presented relatively better quality of life and that at the onset of mid youth age, most TB patients begin to experience less positive PHRQoL. Late age among TB patients however could be understood to be associated with poorer rating of the PHRQoL.

This finding parallels findings of a study conducted by Adeyeye *et al* [11] where lower age groups presented higher PHRQoL as compared to the elderly. Given an assumption that the quality of life as rated by the study participants was related to the TB, it can then be understood that effects of TB on the quality of life is experienced differently among different age groups. It is possible that self-acceptance among TB patients differ across the age groups.

4.3 Level of Education and the Perceived Health Related Quality of Life

The study established that PHRQoL improved positively with the levels of education. The finding may lead an understanding that those with higher levels of education had better PHRQoL. Similar study conducted in Nigeria also established that low levels of education was associated poorer PHRQoL [11]. This finding may be attributed to the higher levels of awareness among individuals with higher levels of education.

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4.4 Marital Status and the Perceived Health Related Quality of Life

Marital status in the study was significantly associated with the PHRQoL. Individuals who were separated had the highest PHRQoL. On the contrast, a study conducted in Nigera [11] found out that respondents without spouses (single, separated or widowed) had higher PHRQoL. This the scholars attribute to the possibility of autonomous lifestyle without their spouses. The findings of this study could be attributable to the fact that only female respondents indicated that they were separated and that in generally, women rated their qualities of life higher than men. The finding that married respondents also had higher rating for the PHRQoL could be explained by the availability of social support within marriage arrangement as opposed to the single respondents who had the lowest rating of the PHRQoL.

4.5 Occupation and Perceived Health Related Quality of Life

The findings of the study indicate that respondents with agriculture as their primary occupation had the highest PHRQoL. Other studies link certain formal occupations with higher PHRQoL [12,13]. The findings of this study however could be attributed to food availability and nutrition which are often associated with better disease management and prognosis. It could also be reasoned that steady source of income as evidenced by higher PHRQoL among those with formal employment as opposed to students with lowest rating influence the PHRQoL.

4.6 Household Size and Perceived Health related Quality of Life

The study established higher PHRQoL among households with 7 and above household members. This finding may lead to an understanding that households with larger membership have higher PHRQoL. This finding could be explained by the possibility that larger household sizes could also be associated with higher income levels and thus higher rating of PHRQoL. However, respondents with 5-6 household members also had least PHRQoL. This finding mirrors the converse for the former explanation.

5. Conclusion

The study concludes that demographic factors are significantly associated with the PHRQoL. Such factors include age, levels of education, marital status, primary occupation and household size. It is therefore evident from the study that TB patients' rating of their PHRQoL depends on their demographic characteristics. From this therefore, it can be understood that indirect effects of TB on the PHRQoL differs based on demographic factors

6. Recommendations

The Study recommend studies to determine the direct association between health effects of TB on the PHRQoL among TB patients. Future studies could also be based on clinical evidence so as to inform intervention deigns.

References

[1] World Health Organization. Tuberculosis Report, 2019. Retrieved 2019, from: https://www.who.int/news-room/fact-sheets/detail/tuberculosis

[2] World Health Organization. Global tuberculosis report 2013.

[3] Salehitali, S., Noorian, K., Hafizi, M., & Dehkordi, A. H.. Quality of life and its effective factors in tuberculosis patients receiving directly observed treatment short-course (DOTS). Journal of Clinical Tuberculosis and Other Mycobacterial Diseases, 2019, 15: 100093.

[4] Martz, E. (Ed.). Promoting self-Management of chronic health conditions: theories and practice. Oxford University Press, 2017.

[5] Ramos-Goñi, J. M., Pinto-Prades, J. L., Oppe, M., Cabasés, J. M., Serrano-Aguilar, P., & Rivero-Arias, O.. Valuation and modeling of EQ-5D-5L health states using a hybrid approach. Medical care, 2017, 55(7): e51-e58.

[6] Haghi, H. B., Hakimi, S., Mirghafourvand, M., Mohammad-Alizadeh, S., & Charandabi, M. F.. Comparison of Quality of Life Between Urban and Rural Menopause Women and its Predictors: A Population Base Study. International Journal OF Women Health and Reproduction Sciences, 2017, 5(2): 137-142.

[7] Zhang, L., Xia, Y., Zhang, Q., Fu, T., Yin, R., Guo, G., ... & Gu, Z.. The correlations of socioeconomic status, disease activity, quality of life, and depression/anxiety in Chinese patients with rheumatoid arthritis. Psychology, health & medicine, 2017, 22(1): 28-36.

[8] Juni, M. H., & Syafiq, S. S.. Systematic Review of Determinants of Health-Related Quality of Life (HRQoL) Affecting Well-being of Pulmonary Tuberculosis (PTB) Patients. International Journal of Public Health and Clinical Sciences, 2019, 6(4): 1-17.

[9] Louw, J., Peltzer, K., Naidoo, P., Matseke, G., Mchunu, G., & Tutshana, B.. Quality of life among tuberculosis (TB), TB retreatment and/or TB-HIV

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co-infected primary public health care patients in three districts in South Africa. Health and quality of life outcomes, 2012, 10(1): 77.

[10] Genga, E. K., Otieno, C. F., Ogola, E. N., & Maritim, M. C.. Assessment of the perceived quality of life of non insulin dependent diabetic patients attending the Diabetes Clinic in Kenyatta National Hospital. IOSR J Pharm, 2014, 4(3): 15-21

[11] Adeyeye, O. O., Ogunleye, O. O., Coker, A., Kuyinu, Y., Bamisile, R. T., Ekrikpo, U., & Onadeko, B.. Factors influencing quality of life and predictors of low quality of life scores in patients on treatment for pulmonary tuberculosis: a cross sectional study. Journal of public health in Africa, 2014, 5(2).

[12] Duyan, V., Kurt, B., Aktas, Z., Duyan, G. C., & Kulkul, D. O.. Relationship between quality of life and characteristics of patients hospitalised with tuberculosis. The International Journal of Tuberculosis and Lung Disease, 2005, 9(12): 1361-1366.

[13] Muhammed, S., Nagla, S., & Morten, S.. Illness perceptions and quality of life among tuberculosis patients in Gezira, Sudan. African health sciences, 2015, 15(2): 385-393.

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ARTICLE

Factors Affecting Compliance towards Radiation Protection Equipment among Radiographers: A Cross Sectional Study

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Radiation protection equipment

ABSTRACT

Background: Radiographers were at risk of x-ray radiation. Ministry of Heath of Indonesia made a regulation act no. 33 year 2007 to secure radiographers on ionizing radiations by using radiation protection equipment (RPE). Objective: This study identified the factors affecting compliance towards RPE among radiographers and determined the correlation between influencing factors and compliance towards RPEs. Methods: The study conducted a quantitative descriptive-correlational design in a cross sectional approach. A total of 103 radiographers answered the online self administered questionnaires from 9 government hospitals at Jakarta, Indonesia. Results: It was identified that personal factors were the knowledge and motivations while the availability of RPEs and standard operating procedures were environmental factors. The knowledge (p=0.001, r=0.321) and motivations (p=0.018, r=0.232), and availability of RPE (p=0.138, r=0.146) and standard operating procedures (p=0.023, r=0.224) were factors affecting a compliance to RPEs. It was however determined that gender (p=0.251, r=0.113), and place of work (p=0.479, r=0.070) were not correlated to both personal and environmental factors. On the contrary, age (p=0.031, r=0.212), highest educational attainment (p=0.039, r=0.203), years of experience (p=0.001, r=0.336), and training (p=0.001, r=0.341) influenced both personal and environmental factors affecting compliance of radiographers towards RPEs. Findings: It was found that Dr. Cipto Mangunkusumo (p=0.271), Persahabatan (p=0.133), Fatmawati (p=0.357), Otak Nasional (p=0.238), Pasar Rebo (p=0.356), Tarakan (0.255), and Koja (p=0.199) hospitals were not probable to comply towards RPEs. Only Infeksi Sulianti Suroso (p=0.21), and Budhi Asih (p=0.0002) hospitals were most probable to comply towards RPEs.

1. Introduction

1.1 Background

Globally, radiographers should have protection from radiation exposure before, during, and after any radiographic examinations [1]. Working with radiation can cause tissue damages or genetic abnormalities - the stochastic effects [2]. However, each radiation contains certain risks, so only unnecessary exposures should be avoided and should be kept as low as possible [1,2]. Radiation protection (RP) is a fundamental radiation safety practice that remains important when performing

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radiography, not only among radiographers but also from patients to minimize the risk of stochastic effects [2].

Many hospitals worldwide uses RPEs as an early protective measure against radiation hazards [3]030; (2=8.683. The use of RPE in Ohio Department of Health has extensively been advocated during radiological procedures, because the absorbed radiation dose can be reduced as much as 99.4% following to use of (1 mm) lead shield [4]. In Adam Malik Hospital, Medan there are 81.8% of radiographer's who are non-compliant to the use of RPEs, and 13.6% of radiographers' dose to radiation exposure are above the average [5].

In Indonesia, in four hospitals in Semarang showed a result of 96.8% of radiographers with non-compliance to RPE [6]. mentioned that in some hospital in Pekan Baru from 2008 to 2011 had 10.3% of radiographers whose leukocyte levels are abnormal because of non-compliance towards RPE [2].

The Ministry of Health (MOH) of Indonesian made a regulation act no. 33 year 2007 about the safety and security of radioactive sources and ionizing radiations that aims to ensure the safety and protection among workers, and patients [7]. To promote the level of RP, the radiology department should utilize some equipment such as lead aprons, glasses, gloves, gonad shields, thyroid shields, and radiation area signs also called radiation protection equipment (RPEs)[8-10].

1.2 Problem Statement

In Indonesia compliance towards the use of RPEs was also a problem especially among radiographers [11]. Although the hospitals in Indonesia have been providing RPEs, enforcing approved regulations for radiographers to use is not emphasized [9].

Poor compliance with safety practices and using RPEs among radiographers will lead to stochastic effects [10]. There is a correlation between knowledge, attitude, behavior, training, and counseling towards the use of RPE [12] and that is why compliance levels on using RPEs should be reiterated [13,14]. In addition, radiographers need to be aware on their roles in ensuring total compliance towards RPEs in their institutions [15]. Lack of compliance are the factor of age, education, training, motivation and duration of work, hence the need for a cross sectional study [16]003.

1.3 Significant of Study

The findings of this study will give the positive effect to the Indonesian society of radiographers as new knowledge in identifying and determining RPE compliance. The greater will the demand be for radiographers as the safety on RPE compliance is achieved [17]. In addition, the application of the principles of RP will also be significant to the training institutions, as it paves new ways in changing the radiographers' safety culture [18].

1.4 Objectives

Specifically, it is hoped to:

(1) Identify factors affecting compliance of radiographers towards RPEs.

(2) Determine the correlation between influencing factors and compliance towards RPEs.

1.5 Operational Definition

The demographic data are the gender, age, education, workplace, year of experience, training among RP officers as influencing variables affecting the factors such as knowledge, motivations, availability of RPEs, and standard operating procedures are all operationally defined on table 1.

Table 1. Operational Definition

Variables	Definition	Criteria
Compliance	The adherence on the use of RPEs	1. High (If Score total≥ median) 2. Low (if score total<mean)
Gender	Male or female	1. Male 2. Female
Age	Births in years	1.<40 years old 2.≥40 years old
Education	Formal highest education received	1. <Bachelor 2. ≥Bachelor
Place of Work	Place where radiographers do radiographic examinations	1.Centres Hospitals 2.District Hospitals
Year of experience	Number of years employed as radiographer	1.<10 years 2.≥10 years
Training	The availability of training in radiography	1. No 2. Yes
Knowledge	Personal factor of the radiographers as how they know much about / on compliance towards RPEs	1. High (If Score total≥ mean) 2. Low (if score total<mean)
Motivations	Personal factor of radiographers addressing attitudes to be willing to use RPEs while working	1. Positive (if score total ≥ mean) 2. Negative (if score total<mean)
Availability of RP Equipment	Environmental factor on the availability of RPEs in radiology unit	1. Complete (If Score total≥ mean) 2. Not Complete (if score total<mean)
Standard Operating Procedures	Environmental factor on the procedures addressing practice of radiographers to use the RPEs	1. Available (If Score total≥ mean) 2. Not Available (if score total<mean)

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2. Methods

2.1 Research Design

A descriptive-co relational quantitative cross-sectional design was used. This is the most appropriate design for analyzing the relationship between the dependent and independent variables in a specific point in time[19].

2.2 Research Location

The study was conducted at 9 government hospitals in Jakarta, from 5 Centre Hospitals such as (1) Dr. Cipto Mangunkusumo, (2) Persahabatan, (3) Fatmawati, (4) Sulianti Suroso, (5) Otak Nasional, and from 4 district hospitals such as (6) Pasar Rebo, (7) Budhi Asih, (8) Tarakan, and (9) Koja.

2.3 Population and Sampling Technique

A total of 152 radiographers at Jakarta were selected working with government hospitals. Of the 152, only 103 responded. The inclusion and exclusion criteria using a snowball sampling technique were as follows.

2.4 Inclusions and Exclusions

The inclusion criteria in selecting respondents are: (1) radiographers at least a diploma graduate and (2) working period of at least 2 years. The exclusions criteria are (1) radiographers who do not use x-ray machines, magnetic resonance imaging, ultrasound, and nuclear radiotherapy, and (2) practicing radiographers who were newly employed in less than 6 months.

However, in order to mathematically determine the overall sample size from the 9 hospitals, we used the formula found on box 1 [20].

Box 1. Formula for determining the overall sample size

$$n = \frac{N}{1 + N(d)^2}$$

Description:

n : number of samples

N : Number of Populations

d : level of error 5% (0.05)

n =103 radiographers

2.5 Sample Size

The distribution of the respondents can be seen in table 2 using the inclusion and exclusion criteria.

Table 2. Respondents

No	Name of hospitals	Population	Respondents
1	Dr. Cipto Mangunkusumo	36	25
2	RSUP Persahabatan	25	14
3	RSUP Fatmawati	11	8
4	RSUP Infeksi Sulianti Suroso	8	6
5	RSUP Otak Nasional	17	11
6	RSUD Pasar Rebo	11	7
7	RSUD Budhi Asih	14	10
8	RSUD Tarakan	12	9
9	RSUD Koja	18	13
	Total	152	103 radiographers

2.6 The Questionnaire

We used an online questionnaire. The careful design of the questions is critical and can eliminate bias when it is delivered online. The questionnaire was distributed to 9 government hospitals in Jakarta to be answered by the n103 radiographers (table 2).

The first part asked of the demographic profile of the respondents such as their gender, age, education, workplace, year experience, and training on RPs. Respondents must answer a 4-point scale with 10 questions about their knowledge on RPs in the second part. The third part covered 10 questions regarding the motivations of the respondents on RPEs. The fourth part was 10 questions about availability of RPEs. The last part was a 10 question about the standard operating procedures on RPEs.

Questions on compliance towards RPEs among radiographers were generated and reconstructed from Hubungan Antara Pengetahuan Tentang Resiko Potensi Bahaya Radiasi Dan Kepatuhan Penggunaan Alat Pelindung Diri Pada Pekerja Radiasi Di Bagian Radiologi [21], Tingkat Kepatuhan Mahasiswa Profesi Dalam Proteksi Diri Terhadap Paparan Radiasi Di Bagian Radiologi Dental Rumah Sakit Gigi Dan Mulut Pendidikan Universitas Hasannudin [1], Factors Related To Radiation Safety Practices In California [14], Analisis Faktor Yang Berhubungan Dengan kepatuhan Menggunkan Alat Proteksi Diri [22] as a reference to the current study.

2.7 Data Analysis

In this study the SPSS version 21 was used to analyze data. Descriptive statistics of frequencies and percentages were displayed. In the analysis of variables, chi square was also used. Coding for questions was done, taking careful consideration into achieving inter-coder reliability and resulting in a standardized coding. A small scale pilot

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study was used before a full scale research was done to validate the reliability of the generated and reconstructed online self administered questionnaire.

2.7.1 Pilot Study

A pilot study was conducted from 10% of the formulated sample size. The respondents from the pilot study were no longer used in the full scale research. The inclusion and exclusion criteria were also implied upon selecting pilot respondents. The results from the pilot study enabled the researcher to detect areas requiring further improvement, and to further detect areas of clarity and precision to achieve the objectives set.

2.7.2 Validity

To test whether the instrument used is valid, we calculated the correlation coefficient alpha between each value on the question number with their total value. Furthermore, we tested the significance of the questionnaire comparing it with the r table. When t count > t table or r count > r-table, then the questions were valid [23]. The formula is found below:

$$r_{XY} = \frac{N\sum XY - (\sum X)(\sum Y)}{\sqrt{\{N\sum X^2 - (\sum X)^2\}\{N\sum Y^2 - (\sum Y)^2\}}}$$

Information:
r = Product Moment Correlation
X = Score Statement
Y = Total Score entire statement
XY = Score statement multiplied by the total score
N = Number of respondents pretest
Criterion validity of a question can be determined if:
a. r count> r table, then the questions on the questionnaire was valid.
b. count r <r table, then the questions on the questionnaire was not valid.

2.7.3 Reliability

Reliability is an index indicating the extent to which a measuring instrument can be trusted. This means showing how far these measurements are consistent when measurements are made twice using the same measuring instrument.

The use of Cronbach's alpha formula below will test the reliability of the questionnaire if it has a value above 0.7 [24].

$$r_{11} = \left(\frac{n}{n-1}\right)\left(1 - \frac{\sum {\sigma_1}^2}{{\sigma_t}^2}\right)$$

Information :
r11 = reliability was sought
$\Sigma\sigma 12$ = total variance score for each item
$\sigma t2$ = total variance
This was interpreted [25] in the following: 0.00 to 0.20 less reliable, having value 0.21 to 0.40 rather reliable, the value 0.41 to 0.70 quite reliable, while the value 0.71 to 0.90 reliable, and finally the value 0.91 to 1.00 is very reliable.

2.7.4 Univariate Analysis

The results of this analysis are presented in tables. The univariate analysis was distributed using frequencies, probabilities, mean, standard deviations, and percentages of each of the variables [19].

2.7.5 Bivariate Analysis

Pearson correlation was used for determining the strength of the relationship between influencing factors and compliance [23]. Here are the guidelines to provide interpretation and analysis for the correlation coefficient [25,26]:

(1) None or weak if the correlation coefficient is less than 0.10
(2) Low if the correlation coefficient value is between 0.10-0.29
(3) Moderate if the correlation coefficient value is between 0.30-0.49
(4) Strong if the correlation coefficient value is between 0.50-0.69
(5) Very Strong if the correlation coefficient value is between 0.70-0.89

The correlation coefficient (r) and significant value (*p*) will be calculated. To perform correlation test, we obey the 5 criteria [25]:

(1) Data must be in paired
(2) Quantitative data
(3) Normal distributed data
(4) Two variables data must be linear
(5) Two variables data must be homoscedastic.

That is why normality, linearity, and homoscedasticity tests were done before inferential analysis. Shapiro-Wilk test was also done to prove that the data is within $p<0.05$. However, Shapiro-Wilk test sometimes maybe over-sensitive until false positively interpreting the data that is not normal in distribution. Therefore, two more additional statistical test: skewness & kurtosis was used to double confirm the results of the Shapiro-Wilk test between -1.96 until 1.96 [25]. Table 3 summarizes the data analysis.

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Table 3. Data Analysis

Research objectives	Questionnaire	Analysis
1. Identify factors affecting compliance of radiographers towards RPEs.	**A. Personal factors questions with scale 4** 1.Knowledge:1.very irrelevant,2.irrelevant,3.relevant, 4.very relevant 2.Motivation: 1. Influenced by colleagues, 2.not reprimanded by supervisor, 3.follow the rules, 4.to be safe. **B. Environmental factors questions with scale 4** 1. Availability equipment: 1.very unfeasible, 2. Unfeasible, 3.feasible, 4.very feasible. 2.Standard Operating Procedure: 1.strongly disagree,2.disagree,3.agree,4. strongly agree	Univariate Analysis: Frequencies and Percentile ranking
2. Determine the correlation between influencing factors and compliance towards RPEs.	**Demographic Factors:** 1.Geneder:: Male/Female 2.Age ≥40 yo/<40yo 3.Education: ≥Bachelor/<Bachelor 4.Workplace: Centre Hospitals/District Hospitals 5.Year experience ≥10/<10 6.Training: Yes/No **A. Personal factors:** 1.Knowledge High/Low 2.Motives: Positive/Negative **B. Environmental factors:** 1.Availibiality RPE Complete/Not Complete 2.Attitude regard SOP: Good/Not Good	1.Chi square test, significant if P value <α 0.05,and 2.Pearson correlation for determining the strength of the relationship between independent & dependent variables i. None or weak if the correlation coefficient is less than 0.10 ii. Low if the correlation coefficient value is between 0.10-0.29 iii. Moderate if the correlation coefficient value is between 0.30-0.49 iv. Strong if the correlation coefficient value is between 0.50 or -0.69 iv. Very Strong if the correlation coefficient value is between 0.70 or -0.89

2.8 Ethics

After researchers acquired permission from the chief radiographers from the 9 hospitals, ethical permission was also acquired from the Ministry of Health, Indonesia for use of their government hospitals. Permission was also given by Lincoln University College, Malaysia for academic purposes. Respondents were given the freedom to decide whether or not to participate in voluntary research. The researchers gave an explanation to the respondents about the purposes and the benefits. The consent was online and explained to the surveyed radiographers who met the inclusion criteria. The consent incorporates the research title as well as the benefits so that the respondent understands the purpose and aims of the research. Researchers did not include the respondent's name on the data collection sheet, but only given certain code in order to keep the identity of the respondents confidential. The respondents' email addresses from the online survey were also kept confidential.

3. Results

3.1 Identify Factors Affecting Compliance of Radiographers towards RPE

It was however identified that knowledge (p=0.001, r=0.321) and motivations (p=0.018, r=0.232), and availability of RPEs (p=0.138, r=0.146) and standard operating procedures (p=0.023, r=0.224) were factors affecting a compliance to RPEs.

The personal factors are the knowledge and motivations. The availability of RPEs and standard operating procedures are environmental factors.

3.2 Determine the Correlation between Influencing Factors and Compliance towards RPE

The demography as influencing factors are gender, age, highest educational attainment, place of work, years of experience, and training found on Table 4.

It was determined that gender (p=0.251, r=0.113), and place of work (p=0.479, r=0.070) were not correlated to both personal and environmental factors. On the contrary, age (p=0.031, r=0.212), highest educational attainment (p=0.039, r=0.203), years of experience (p=0.001, r=0.336), and training (p=0.001, r=0.341) influenced both personal and environmental factors affecting compliance of radiographers towards RPEs.

It is however interesting to determine that there is no significant correlation between knowledge as a personal factor and availability of RPEs as an environmental factor (p=0.138; r=0.146). On the other hand, a weak correlation was determined between standard operating procedure as an environmental factor and motives as a personal factor affecting compliance towards RPEs (p=0.023 and r=0.224); and between motives as a personal factor and availability of RPEs as an environmental factor (0.018 and

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Table 4. Compliance on RPE

No	Influencing factors	Compliance		Non Compliance		N	%	r time	P Value	OR (95%CI)
		n	%	n	%					
1. Gender										
	Male	28	520.8%	25	470.2%	53	100			0.630
	Female	32	640.0%	18	360.0%	50	100	-0.133	0.251	0.286-1.388
	Total	60	580.3%	43	410.7%	103	100			
2. Age										
	≥40	23	74.2	8	25.8	31	100			2.720
	<40	37	51.4	35	48.6	72	100	0.212	0.031	1.075-6.878
	Total	60	58.3	43	41.7	103	100			
3. Education										
	≥ Bachelor	11	84.6	2	15.4	13	100			4.602
	< Bachelor	49	54.4	41	45.6	90	100	0.203	0.039	0.964-21.960
	Total	60	58.3	43	41.7	103	100			
4. Workplace										
	Centre Hospitals	39	60.9	25	39.1	64	100			1.337
	District Hospitals	21	53.8	18	46.2	39	100	0.070	0.479	0.598-2.992
	Total	60	58.3	43	41.7	103	100			
5. Experience										
	≥10 years	31	79.5	8	20.5	39	100			4.677
	< 10 years	29	45.3	35	54.7	64	100	0.336	0.001	1.864-11.735
	Total	60	58.3	43	41.7	103	100			
6. Training										
	Yes	26	83.9	5	16.1	31	100			5.812
	No	34	47.2	38	52.8	72	100	0.341	0.001	2.007-16.826
	Total	60	58.3	43	41.7	103	100			
7. Knowledge										
	High	43	71.7	17	28.3	60	100			3.869
	Low	17	39.5	26	60.5	43	100	0.321	0.001	1.687-8.872
	Total	60	58.3	43	41.7	103	100			
8. Motives										
	Positive	43	67.2	21	32.8	64	100			2.650
	Negative	17	43.6	22	56.4	39	100	0.232	0.018	1.167-6.018
	Total	60	58.3	43	41.7	103	100			
9. Availability of RPE										
	Complete	34	65.4	18	34.6	52	100			1.816
	Not Complete	26	51.0	25	49.0	51	100	0.146	0.138	0.822-4.011
	Total	60	58.3	43	41.7	103	100			
10. Standard operating procedure										
	Good	40	67.8	20	32.2	59	100			2.526
	Not Good	20	45.5	24	54.5	44	100	0.224	0.023	1.128-5.695
	Total	60	58.3	43	41.7	103	100			

r=0.232). On the contrary, a strong correlation was determined between it was determined between knowledge as a personal factor and standard operating procedures as an environmental factor (p=0.001 and r=0.321) towards compliance to RPEs.

3.3 Findings

Of the 10 hospitals, compliance towards RPE was not probable among 8 hospitals found on table 5. Only 2 hospitals have more probability to comply.

Table 5. Compliance towards RPE per hospitals

	(N)	mean	Standard deviation	p
Dr. Cipto Mangunkusumo	25	1.28	0.458	0.271
RSUP Persahabatan	14	1.57	0.514	0.133
RSUP Fatmawati	8	1.13	0.354	0.357
RSUP Infeksi Sulianti Suroso	6	1.83	0.408	0.021
RSUP Otak Nasional	11	1.36	0.505	0.238
RSUD Pasar Rebo	7	1.14	0.378	0.356
RSUD Budhi Asih	10	1.90	0.316	0.002
RSUD Tarakan	9	1.33	0.500	0.255
RSUD Koja	13	1.42	0.496	0.199

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Dr. Cipto Mangunkusumo (p=0.271), Persahabatan (p=0.133), Fatmawati (p=0.357), Otak Nasional (p=0.238), Pasar Rebo (p=0.356), Tarakan (0.255), and Koja (p=0.199) hospitals were not probable to comply towards RPE.

Infeksi Sulianti Suroso (p=0.21), and Budhi Asih (p=0.0002) hospitals were most probable to comply towards RPE.

4. Discussion

4.1 Strengths, Weaknesses, and Limitations

The strength of this study is its design. A combination of a descriptive-correlation is strong since it does not only describe the findings of the variables but assumes relationship. In that way, the result may be used in the real settings. Lastly, the strength of this design is the use of the online survey questionnaire. This is less stressful where time and financial constraints are factors to be considered[25] such as printing of the questionnaires. In addition, survey is less complex to modify, adapt, or adopt.

The weakness of this study is the vast number of confounding variables – the age, gender, years experience, educational attainment, training and the work place of the respondents influencing the identified factors affecting compliance towards RPEs. That is why this study could not be generalized and should be taken with caution if it were to be used as reference because the culture is only limited in a given point in time. Finally, a quantitative survey design is also a weakness if the respondents are not sincere and truthful of answering the questions. To assume maturity of the behavior and relationship of the quantified results, the survey questions are further discussed.

4.2 Discussion of Demographic Influencing Factors

4.2.1 Gender

The differences in values and traits by gender usually affect decision-making[27]. Men will compete for success and are more likely to ignore the existing rules because they perceive achievement as a competition, while women focus more on performing tasks well in line with the prevailing rules and maintaining harmonious working relationships [6,28]. There were more male respondents from hospitals who were low in compliance as compared with the females.

On the contrary, the gender concept, in terms of knowledge, experience, and behavior towards compliance, both men and women have the same potential in accordance with the efforts undertaken [28]. That is why age was also used in addition to gender to determine correlation towards compliance.

4.2.2 Age

Increasing age is more able to show the maturity and capability of rational thinking, and ability to control the emotions [29]. Also, adults are the more tolerant in views and behaviors that is different from intellectual and psychological maturity [30]. A person will experience a decline in mental function as they grow older, so the ability to absorb knowledge and understand important implications of policies also decreases [31]. Hospitals that have low compliance have respondents who are ≥40 years old.

This statement is different with who mentioned that the level of work performance improves with increasing age[32]. That is also why some hospitals that have low compliance have respondents who are ≤40 years old.

This is why in addition to age and gender, it is also interesting to correlate compliance with highest educational attainment.

4.2.3 Education

Education determines the extent of a person's knowledge as being able to find their own problem-solving in the workplace and someone with low education is very difficult to accept the concept of change[30]. Education is a development in which staff gains knowledge and skills for positive purposes which is essential for its performance in terms of cognitive, psychomotor, and attitude[33]. The educational background affects the application of patient safety[34]. This is why hospitals who has low compliance have respondents with <bachelor's degree.

On the contrary stated that there is no significant relationship between education and compliance to patient safety[6]. That is why hospitals that have low compliance also have respondents who have ≥bachelor's degree.

This is why it is interesting to add the workplace environment as a significant factor affecting compliance in addition to age, gender, and highest educational attainment.

4.2.4 Workplace

The work place that is everything that surrounds the workers that can affect in carrying out tasks embedded[35]. if the employee enjoys the workplace environment the activities are used effectively and performance is also high [36]. The workplace environment includes working relationships formed between fellow employees and working relationships between subordinates and superiors in which employees enjoy[14].

This is quite inconclusive to determine why hospitals had low compliance. That is why years of work experience is also included in the demographic analysis.

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4.2.5 Years of work experience

The work experience is already known a factor of a workplace environment that can influence the person to behave because it an employee can recognize patterns that tends to recur, also added that the experience factor can influence the person to comply with policies and regulations of the organization [37]. That is why hospitals with lower compliance have respondents working <10 years.

On the contrary, the longer the work period is, will make the workers more complacent with the compliance to work conditions. If worker is familiar with the workplace and the dangers of work, the compliance is lower [30]. That is also why hospitals with low compliance have respondents working ≥10 years.

That is why it is noteworthy to include trainings and continuous professional developments which the respondents have achieved affecting compliance, in addition to age, gender, highest educational attainment, workplace, and years of experience.

4.2.6 Training

The continuous trainings can form a safe behavior [38]. The training is conducted when workers do not know how to work safely [37]. Giving training can benefit the workers to increase the likelihood to improve their compliance[6]. Most of the respondents from the hospitals have not acquired continuous trainings which affected their compliance to policies.

4.3 Discussion of Personal Factors

4.3.1 Knowledge

Knowledge is a factor in the person as a component that will influence compliance[37]. Inadequate knowledge about the risks and dangers and accidents would allow workers to be indifferent and may act unsafe or detrimental to the safety of themselves[39].

Increased knowledge does not always lead to changes in behavior. Knowledge is something that needs to be a strong factor so that one acts with critical thinking[38]. Knowledge from the personal side comes from cognitive conscience[37]. Knowledge of radiographers regarding optimal techniques, radiation dose, RP measures are important for reducing radiation exposure[40]. The 9 hospitals in this study that has respondents with lesser training have lesser knowledge leading to a low compliance.

4.3.2 Motivations

A person's motivations addresses attitudes[37]. The individual's motivation does not lie in a series of movers, but rather focuses on the hierarchy, a particular "higher" need to expand the "lower" and unsatisfied needs[41]. Motivations on execution of performances will require the fighting spirit to be high[42]. Performance comes from higher motives[37].There are two ways to improve work motives: 1) Being hard, by forcing worker to work hard or by giving rewards. 2) Provide meaningful goals[43].

The 9 hospitals in this study that has respondents with negative motivations have low compliance.

4.4 Discussion of Environmental Factors

4.4.1 Availability of RPE

Availability of resources is a factor in the environmental components that influence compliance [37].

On the contrary, said that there is no effect between the availability of RPE with compliance [6]. The most of diagnostic equipments in government hospital were obsolete, majority the compliance strictly is on the use of thermoluminiscent dosimetry (TLD) as monitoring equipment [15].

The availability of RPE in this case is one form, where some workers may refuse to use RPE because it causes discomfort and adds the burden of stress on the body[44]. That is why more respondents were not probable to comply.

4.4.2 Standard Operating Procedures

The standard operating procedure addresses the practice [45]. The standard operating procedures are written documents of standards, norms, and policies for expected practice. Standard operating procedures is a factors in an environment that influences compliance [37]. The company must have clear standard operating procedures about the implementation of occupational safety [46]. Respondents who did not comply were mostly affected by unclear standard operating procedures.

5. Recommendations

Moreover, the impact of radiation is often long-term, so it is easily overlooked. Therefore the recommendations are:

(1) Hospitals' management should view safety as an integral part of a strategy for controlling radiation risk, forming a safety [18]. Considering the demographic, personal, and environmental influencing factors will be helpful in including with the strategic management towards RPE compliance.

(2) The MOH in Indonesia should not only provide RPEs on their government-owned hospitals, but should also enhance radiographers' compliance by giving incentives and rewards [9]. The MOH of Indonesia should

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conduct an evaluation on each radiographer from their hospitals to ascertain whether each of their radiological examinations is in compliance with the standard operating procedures.

(3) Refresher courses, continuous educational programs, and trainings to radiographers should be projects at large [47]. The most basic stage is to raise the awareness of radiographers on compliance towards RPE by establishing knowledge on safety culture. The management of the district hospitals should increase the completeness of the RPE facility and require its use by increasing the trainings of the use of such RP equipment so that radiographers comply.

References

[1] Moehadi AST. Tingkat Kepatuhan Mahasiswa Profesi dalam Proteksi Diri terhadap Paparan Radiasi (di bagian Radiologi Dental Rumah Sakit Gigi dan Mulut Pendidikan Universitas Hasanuddin) SKRIPSI, 2015. Http://RepositoryUnhasAcId/Handle/123456789/14235

[2] Mayerni, Ahmad A, Abidin Z. Dampak Radiasi Terhadap Kesehatan Pekerja Radiasi Di Rsud Arifin Achmad, Rs Santa Maria Dan Rs Awal Bros Pekanbaru. J Ilmu Lingkung, 2013, 7: 114–27.

[3] Faniah AM. Faktor-Faktor Yang Berhubungan Dengan Kepatuhan Penggunaan Apd Earplug Dan Sarung Tangan Pada Pekerja Unit Perbaikan DI PT. Kai Daop VI Yogyakarta Dipo Solo Balapan. Http://EprintsUmsAcId 2016

[4] Karami V, Zabihzadeh M, Gilavand A. Survey of the use of X-ray beam collimator and shielding tools during infant chest radiography. Int J. 2016.

[5] Wirjosentono B. Kepatuhan Radiofrafer Pada Pelaksanaan Peraturan Keselamatan Dan Kesehatan Kerja Unit Radiologi Dan Pengaruhnya Terhadap Gangguan, 2008.

[6] Hendra Y, Utomo M, Salawati T. Beberapa Faktor Yang Berhubungan Dengan Praktik Pemakaian Alat Pelindung Diri (APD) Pada Radiografer DI INSTALASI Radiologi 4 Rumah Sakit DI Kota Semarang. J Kesehat Masy Indones 2017;7.

[7] Rahmawati N, Martiana T. Analisis Safe Behacior Dengan Pendekatan Behavior-Based Safety Pada Radiografer DI Rumah Sakit Dr. Soetomo Surabaya, 2014.

[8] Rostamzadeh A, Farzizadeh M, Fatehi D. Evaluation of the Level of Protection in Radiology Departments of Kermanshah, Iran, 2015, 12: 200–8. https://doi.org/10.22038/ijmp.2015.6221

[9] Yusri H, Situmorang J. Sikap Terhadap Keselama Tan Dari Pekerja Radiasi Rumahsakit Dan Industri Indonesia. Bul Keselam Statut 2000, 1: 8–14.

[10] Magbojos CR, Flores N, Manalo JC, Hernandez EG. Quality assurance practices among radiologic and x-ray technologists at Oriental Mindoro, Philippines. THE STETH, 2011, 5.

[11] Khoiri M. Upaya Peningkatan Budaya Keselamatan Pekerja Radiasi Rumah Sakit di Indonesia. Semin Nas VI SDM Teknol Nukl, 2010.

[12] Hakim L. Penggunaan Alat Pelindung Diri (APD) Oleh Pekerja Radiasi Pada Instalasi Radiologi Rumah Sakit Di Wilayah Kota Palembang Tahun 2004, 2004.

[13] Andersson B, Fridlund B, Elgán C. Radiographers' areas of professional competence related to good nursing care. J Caring, 2008.

[14] Reagan JT, Slechta AM. Factors related to radiation safety practices in California. Radiol Technol. 2010, 81: 538–47.

[15] Adejumo S, Irurhe N, Olowoyeye O. Evaluation of compliance to radiation safety standard amongst radiographers in radiodiagnostic centers in South West, Nigeria. World J Med 2012.

[16] Kasim Y, Mulyadi N, Kallo V. Hubungan Motivasi & amp; Supervisi Dengan Kepatuhan Perawat Dalam Penggunaan Alat Pelindung Diri (Apd) Pada Penanganan Pasiem Gangguan Muskuloskeletal DI Igd Rsup Prof DR. R. D. Kandou Manado. J Keperawatan, 2017, 5.

[17] Bapeten. Peraturan Pemerintah Nomor 33 Tahun 2007 tentang Keselamatan Radiasi Pengion dan Keamanan Sumber Radioaktif - JDIH-BAPETEN. Peratur Pemerintah, 2007, 40. https://jdih.bapeten.go.id/index.php/site/dokview/id/21 (accessed October 10, 2017).

[18] Bapeten. Peraturan Kepala Badan Pengawas Tenaga Nuklir Nomor 4 Tahun 2013 tentang Proteksi dan Keselamatan Radiasi Dalam Pemanfaatan Tenaga Nuklir - Jdih-Bapeten. Perka Bapeten, 2013, 3. https://jdih.bapeten.go.id/index.php/site/dokview/id/322 (accessed October 10, 2017).

[19] Notoatmodjo S. Metodologi Penelitian Kesehatan Jakarta: Rineka Cipta..(2007). Promosi Kesehat Teor Dan Ilmu Perilaku Jakarta, 2002.

[20] Nursalam P. Pendekatan praktis metodologi riset keperawatan. Jakarta CV Sagung Setyo, 2001.

[21] Dwiastuti T. Hubungan Antara Pengetahuan Tentang Risiko Potensi Bahaya Radiasi Dan Kepatuhanenggunan Alat Pelindung Diri Padapekerja RadiasiDI Bagian Radiologi Rs Umum Tangerang. Univeritas Esa Unggul, 2013, 0.

[22] Putri K, AW Y. Analisis Faktor Yang Berhubungan

  DOI: https://doi.org/10.30564/jhp.v1i2.1505

Dengan Kepatuhan Menggunakan Alat Pelindung Diri. J [Serial Online] Http//Journal Unair Ac, 2014.

[23] Sugiyono. Metode Penelitian Administratif 2013. http://zeilaputri.blogspot.co.id/2015/06/perspektif-metode-penelitian.html (accessed January 3, 2018).

[24] Ghozali I. Aplikasi analisis multivariate dengan program SPSS. Badan Penerbit Universitas Diponegoro, 2006.

[25] Kim SY, Park JE, Lee YJ, Seo H-J, Sheen S-S, Hahn S, et al. Testing a tool for assessing the risk of bias for nonrandomized studies showed moderate reliability and promising validity. J Clin Epidemiol, 2013, 66: 408-414. https://doi.org/https://doi.org/10.1016/j.jclinepi.2012.09.016

[26] Allen, Unwin. Surveys In Social Research - David De Vaus - Sixth Edition. Google B, 2014. https://books.google.co.id/books?hl=id&lr=&id=rnxiAgAAQBAJ&oi=fnd&pg=PP1&dq=Survey+in+Social+Research+5th+Edition,+Allen+and+Unwin&ots=6dPIDdquLL&sig=fHfRzqu5fxAmP5IqX8wdaewdELs&redir_esc=y#v=onepage&q&f=false (accessed January 4, 2018).

[27] Hastuti, Sri. Perilaku Etis Mahasiswa dan Dosen Ditinjau Dari Faktor Individual Gender dan Locus ff Control (Studi Empiris Pada Fakultas Ekonomi Universitas X di Jatim). J Ris Ekon Bisnis, 2012, 7: 58–73. https://doi.org/10.1234/JREBIS.V7I1.66

[28] Fakih M. Analisis gender & transformasi sosial. Pustaka Pelajar, 1996.

[29] Siagian S. Manajemen sumber daya manusia, 2014.

[30] Notoatmodjo S. Promosi kesehatan dan ilmu perilaku, 2007.

[31] Helliyanti P. Berhubungan Dengan Perilaku Tidak Aman di Dept. Utility and Operation PT Indofood Sukses Makmur Tbk Divisi Bogasari Flour Mills tahun 2009. Skripsi Depok FKM UI, 2009.

[32] Sari L, Asyik B, Miswar D. Karakteristik Sosial Ekonomi Kepala Keluarga Yang Bertempat Tinggal DI Kelurahan Kaliawi. JPG, Jurnal Penelit Geogr 2014.

[33] Hasibuan A. Urgensi Pendidikan Pemakai Dalam Upaya Peningkatan Kualitas Layanan di Perpustakaan. Dalam J Iqra, 2008.

[34] Ridley R. The relationship between nurse education level and patient safety: An integrative review. J Nurs Educ, 2008.

[35] Reynaldo A, Darmastuti I. Analisis Kualitas Sistem Informasimanajemen Dan Lingkungan Kerjaterhadap Kinerja Karyawan Asuransijiwa Bumiputera 1912 Cabang Pandanaransemarang 2015.

[36] Aslam A, Riani AL, Widodo GP. Pengaruh Perilaku Kerja, Lingkungan Kerja, Dan Interaksi Sosial Terhadap Kepuasan Kerja. J Manag Pharm Pract. 2013, 3: 167–74. https://doi.org/10.22146/JMPF.189

[37] Geller E. The psychology of safety handbook, 2016.

[38] Green L, Kreuter M, Deeds S, Partridge K. Health education planning: a diagnostic approach, 1980.

[39] Cahyani D. Faktor-Faktor yang Berhubungan dengan Perilaku Tidak Aman pada Pekerja Pabrik Billet Baja PT Krakatau Stell, Cilegon, Jawa Barat Tahun 2004. Skripsi Depok FKM UI, 2004.

[40] Ismanto, Che Hassan H. Factors Affecting Radiographers' Compliance with Radiation Protection on All Areas of Hospital Settings Worldwide - A Meta-Analysis. IJIRST –International J Innov Res Sci Technol. 2016, 3: 433–8.

[41] Maslow A. Motivation and personality (; revised by R. frager, J. fadiman, C. McReynolds, & R. cox). NY Harper Row 1987.

[42] Ishak A, Hendri T. Manajemen Motivasi. Book 2003. https://www.bukupedia.com/id/book/id-38-25518/pengembangan-diri-motivasi/manajemen-motivasi.html (accessed January 3, 2018).

[43] Munandar A. Psikologi Industri dan Organisasi. Teori Motivasi dan Aplikasinya, 2001.

[44] Roughton J, Mercurio J. Developing an effective safety culture: A leadership approach, 2002.

[45] Prabowo OS. Analisis Pengaruh Human Relation, Kondisi Fisik Lingkungan Kerja, Dan Leadership Terhadap Etos Kerja Karyawantor Pendapatan Daerah DI Pati, 2009.

[46] Suma'mur P. Higiene Perusahaan Kesehatan dan Keselamatan Kerja, 1996.

[47] Hellings J, Schrooten W, Klazinga N, Vleugels A. Challenging patient safety culture: survey results. Int J Health Care Qual Assur. 2007, 20: 620–32. https://doi.org/10.1108/09526860710822752

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ARTICLE

Stabilizing Effects of Ethanolic Extract of Mastic Gum on Microtubule Polymers: an In Vitro Study

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ABSTRACT

Terpenoids are novel natural products isolated from mastic gum. Mastic gum was obtained from the Pistacia Lentiscus tree. Scientific investigations have documented medical and pharmacological properties of mastic gum such as memory enhancement, antifungal, and antibacterial activities. It was astonishing to study the possible interaction of mastic gum extract with microtubule proteins which are involved in memory and consciousness since the administration of mastic gum is evidenced in the improvement of brain functions. Since a number of studies have demonstrated the effect of microtubule dynamics on mammals' memory, in this study, we investigated the effect of Oxygenated Sesquiterpenes (OST) on microtubule polymerization in vitro. OST was purified from the ethanolic extract of mastic gum. The results revealed that OST induces microtubule polymerization; however, microtubule depolymerization was not affected and fluorometric assays showed conformational changes of tubulin in the presence of OST. We interestingly found that colchicine was unable to inhibit MT assembly in the presence of OST and OST was solely more efficient than the combination of OST with paclitaxel for elevating microtubule polymerization rate. We hope that OST could be a promising agent for memory enhancement and the treatment of neurodegenerative diseases as a novel tubulin-binding compound.

1. Introduction

Pistacia lentiscus belongs to the Anacardiaceae family which is found in the Mediterranean regions [1]. Mastic gum is "well-known" for its terpenoid and essential oil compounds. The major components of mastic gum are consisted of flavonoids, triterpenoids, Oxygenated Sesquiterpenes (OST), phenolic compounds and some essential oils which show antifungal and antibacterial activities, and have been used for the treatment of hypertension, dyspepsia, abdominal discomfort and patients suffering from peptic ulcers [2-6]. This study of mastic gum compounds originated from oriental traditional medicine papers which revealed wide usage of mastic gum for decreasing anxiety and stress [7], memory enhancement [7,8], antioxidant behavior [9] and anti-inflammatory activity [10]. At the other end of the spectrum, it has been shown that microtubule (MT) proteins, which particularly play essential structural and functional roles in brain cells, are involved in the memory system [11]. Therefore interaction

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of OST with MT protein was the focus of this study.

MT proteins are dynamic polymers in all eukaryotic cells which play key roles in a variety of cellular functions. They are composed of tubulin heterodimers including α-tubulin and β-tubulin which are able to be assembled from head to tail through binging to guanosine triphosphate (GTP) [12,13]. MTs are essential proteins performing crucial roles in various cell functions such as mitosis, cell motility and cellular transportation [14]. Axonal MTs are the major internal structures defining the external shape, polarity of the neuron and substance transportation including neurotransmitters [15]. MT stability is promoted by MT-associated proteins (MAPs) which bind to MT polymers and regulate the polymerization [13]. MT ends switch between growing and catastrophic state in vitro as well as in situ; this kind of behavior has been termed as dynamic instability [16]. Agents and drugs interfering with MT polymerization are able to have an impact on MT dynamics and functions [17].

Recent investigations indicated the crucial role of MT protein in the enhancement of memory. In 1993, Qian et al. have demonstrated abnormal MT polymerization of old rats' brains in comparison with the juvenile rats [18]. Other Researches in 2002 showed that the infusion of colchicines into the rat bilateral rostrocaudal location has caused impaired memory function, which was due to the dysfunction of MTs [19]. Subsequent analyses have also confirmed the above-mentioned behavior of MT proteins [11,20]. Since MT dysfunction and instability are prominent in neurodegenerative diseases, e.g. Alzheimer's, MT-stabilizing agents could compensate for the loss of MT organization and function [21]. The effect of crocin on MT polymerization and structure in comparison with paclitaxel was previously reported in our lab indicating the ability of crocin for enhancing MT polymerization and nucleation rate as well as disruption of MT dynamics through acting as a stabilizing agent [22].

In this study, we have been searching for an effective molecular ligand interacting with MT proteins, which could stabilize MT polymers without any toxic effects. Hence, the impact of the ethanolic extract of mastic gum on MT dynamics was examined. At least four different compounds were accordingly isolated from ethanolic extract of mastic gum which revealed the adequate potency to induce MT polymerization in a concentration-dependent manner. Optimization of a series of extracted compounds resulted in obtaining OST, which was selected for further investigation because it displayed a high potential to stimulate MT polymerization. Moreover, we investigated the effects of OST combination with colchicine and paclitaxel on MT dynamics.

2. Materials and methods

2.1 Reagents

Paclitaxel and Colchicine (Aldrich Chemical Co.) were dissolved in DMSO before any usages. Phosphocellulose P11 was obtained from Whatman (Florham Park, USA). All other chemicals were purchased from Sigma Chemical Co. All solutions were prepared with double distilled water and were kept at 4 ºC for further application.

2.2 Preparation and Identification of OST by HPLC and GC-Mass

Pistacia lentiscus was granted by Professor. S. M. Ghaffari, University of Tehran. Its fruits were collected and dried in darkness. The essential oils of mastic gum were obtained by the protocol described by Abidi et al. [23]. Four different compounds were isolated from the ethanolic extract of mastic gum by preparative HPLC. The most effective one was Oxygenated Sesquiterpene (OST) which has been mentioned in the following experiments. A C18 reverse-phase column (150 × 3.9 mm inside diameter) was used with a 4 μm Nova-Pack C18 cartridge (Water, Milford, MA). The mobile phase consisted of HPLC was acetonitrile and was run isocratically at a flow rate of 1 ml/min. The elution was monitored at 308 nm. According to terpenoid elution time, the peaks were collected and OST was identified as $C_{15}H_{24}O_2$ with a molecular weight of 236 Dalton assessed by GC-mass spectrometry described by Ammari et al. [8]. The data was not displayed.

2.3 Tubulin Purification

Tubulin was purified from the sheep's brain through two cycles of temperature-dependent polymerization-depolymerization based on the Miller and Wilson method [24] with slight modifications. The fresh brain was homogenized in PEM buffer (100 mM PIPES, 1 mM EGTA, 2 mM MgSO4, and 1 mM ATP, pH 6.9) and the resulting homogenate was centrifuged for 30 min at 30000 g. The tubulin in the supernatant was polymerized for 45 min with the presence of Mg^{2+}GTP and PMG buffer (100 mM PIPES, 2 mM MgSO4, 1 mM EGTA and 3.4 M glycerol, pH 6.9) was used as the polymerization buffer. MTs were pelleted by centrifugation (120,000g, 45 min), resuspended in PEM buffer 4°C for depolymerization process and then followed by one more cycle of polymerization-depolymerization for further purification. In order to eliminate residual MAPs and other remained impurities, tubulin was subjected to the anion exchange chromatography in a phosphocellulose column P11 using the method revealed by Weingarten et al [25]. Purified tubulin was confirmed by

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Coomassie Brilliant Blue staining of 10% SDS-PAGE and then stored at the liquid nitrogen. The protein concentration was measured using Bradford reagent (Bio-Rad, Hercules, USA) with bovine serum albumin as standard [26].

2.4 Microtubule Polymerization and Depolymerization Assay

The effect of OST on the MT polymerization-depolymerization process was determined by turbidometric assays. Therefore, tubulin was incubated in PEM buffer with a final concentration of 2 mg/ml. Different concentrations of the substrates including OST, Paclitaxel, and Colchicine were consequently added to each sample cuvette. The tubulin assembly process was monitored by measuring the absorbance at 350 nm every 20 seconds at 37ºC after the addition of 1 mM GTP using a spectrophotometer equipped with a temperature controller (Varian, Melbourne, Australia). For disassembly assays, MTs incubated either with OST or alone were kept at 4°C and the absorbance changes were monitored at 350 nm. The depolymerized MT was re-polymerized at 37°C and the process was measured again as described above.

2.5 Fluorescence Spectroscopy

8-anilino-1-naphthalenesulfonic acid (ANS) was used as a fluorophore to examine conformational changes of tubulin in the presence of OST. Tubulin (2 μM) was incubated in the presence of different concentrations of OST at 4 °C for 10 min. ANS (50 μM final concentration) was added to the tubulin-OST solution and incubated again for about 7 min. The emissions were monitored between 450-550 nm following excitation at 380 nm. All experiments were carried out at 4 ºC by Cary eclipse fluorescent spectrophotometer (Varian, Australia) and 2 μM tubulin was used in all measurements.

2.6 Transmission Electron Microscopy (TEM)

In-vitro polymerized MTs with and without OST (10 μM) were fixed on carbon-coated Farmavar-treated copper grids with 200 meshes. Each grid was then negatively stained with 10 μl uranyl acetate 1% solution. Excessive uranyl acetate was withdrawn with filter paper and sample grids were air-dried and observed using an HU-12A transmission electron microscope (Hitachi, Japan).

2.7 Cell Culture

Two human neuroblastoma cell lines, SK-N-MC and SK-N-BE (2), and fibroblast cell line, L929, were used as models for exploring the effects of OST on the brain cells. The SK-N-BE (2) cell line was cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco BRL, Grand Island, NY) supplemented with 10% v/v fetal bovine serum (FBS) (Gibco). SK-N-MC and fibroblast cell lines were grown in RPMI-1640 medium, supplemented with 10% FBS. The cells were maintained in a humidified incubator supplied with 5% CO2 and 95% air at 37°C. For measuring the viability of the cells in the presence of various concentrations of OST, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) colorimetric assay was performed in a 96-well cell culture plate after incubation of the substrates for 24 hours. Therefore, absorbance at 570 nm was determined for each well using Powerwave XS2 microplate spectrophotometer (BioTek, USA). The inhibition concentration (IC50) was calculated by linear regression, performed on the linear zone of the dose-response curve for absorbance readings [27]. Data were calibrated to the appropriate calibration curve.

3. Results

3.1 Effect of OST on Tubulin Assembly and Disassembly in Vitro

Turbidometric assays were done in order to investigate the effect of OST on MT polymerization-depolymerization in vitro. Untreated MTs exhibited a temperature-dependent polymerization-depolymerization cycle as long as Mg^{2+}GTP was added. It is displayed that the addition of OST can promote MT polymerization in a concentration-dependent manner (2-10 μM) at 37 ºC (Figure 1). The re-polymerization assay demonstrated a similar result indicating the enhancement of the re-polymerization process in the presence of increasing concentrations of OST (Figure 2). Therefore, it is can be conluded that OST has no effect on formation of tubulin aggregates and normal assembly-disassembly cycle of tubulin proteins in the presence of highet concentration of OST was observed (Figure 2). Moreover, TEM was applied to visualize the MTs structure treated with OST. Electron micrographs showed that MTs were formed and organized longer in the presence of OST with inflated intensity in comparison with control MT polymers (Figure 3).

We additionally evaluated the combined effect of Paclitaxel and OST on MT dynamics. MT was polymerized in the presence of 0.1 mM Paclitaxel and 10 μM OST to compare the OST effects with Paclitaxel on tubulin assembly. Increased level of MT polymerization in the presence of OST was observed compared to Paclitaxel and a combination of OST and Paclitaxel (Figure 4). However, OST was not able to change the inhibitory effect of Paclitaxel on microtubule depolymerization.

To figure out the possible correlation between OST and

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colchicine sites on MT proteins, polymerization was conducted in the presence of 0.1 mM colchicine and 10 μM OST. The result has interestingly exceeded expectations. Turbidity of tubulin-OST complex rocketed about three-fold more than treatment with the combination of OST and colchicine (Figure 5).

3.2 Effect of OST on MT Dynamics at Steady State

The results revealed that the addition of OST on assembled microtubule solution at the equilibrium phase caused an increase in turbidity and the polymerization process initiated again to reach a new equilibrium state (Figure 6). It was comparable to the result obtained from the polymerization assay (Figure 1).

3.3 Effect of OST on the Tubulin Conformation

To investigate the tubulin structural changes in the presence of OST, ANS fluorescence studies have been done. Tubulin-ANS complex manifests strong fluorescence ability that is extremely and environmentally sensitive. It is used to probe the conformational state of the tubulin and to determine the nature of the interactions [28]. The results revealed that the ANS fluorescence emission of tubulin was increased by an increment of OST concentrations due to the changes in the hydrophobic environment around the tubulin protein (Figure 7).

3.4 The Cell Culture Results

After 24 h incubation of OST with three cell lines including SK-N-MC, SK-N-BE (2) and L929, the viability of them was determined above 80%. The morphological changes have not also occurred (the data was not shown) and the results have displayed no significant toxicity at the concentration ranged from 0.2 to 3.2 μM (Figure 8).

4. Discussion

MT proteins are dynamic polymers encompassing tubulin subunits whose functions and stability are supposedly correlated to brain maintenance especially memory function [29]. The MT dynamicity is essential for neuronal proper activity and protection from neurodegeneration [30]. It is sensitive to various chemical agents targeting tubulin or MT polymers. The primary action of these agents is to bind to specific sites on the tubulin in order to alter the polymerization process. Hence, finding a new stabilizing agent with low neurotoxicity and harmful effects might open a new horizon for the treatment of neurodegenerative diseases [21]. Hence, the effects of ethanolic extract of mastic gum, which some evidence confirms its neuroprotective properties [31], on the tubulin structure and function were evaluated in vitro.

The obtained outcomes indicated that OST could increase the rate of MT polymerization in a dose-dependent manner and could change the MT steady-state equilibrium. The assembly-disassembly process in the presence of OST revealed that no aggregate formation was induced even at high concentrations. TEM images could confirm the results obtained from the turbidimetric assay, which illustrated the abundance of MT polymers with no aggregates formation in the presence of OST compared to the control. MT polymer is composed of an unstable tubulin-GDP core and a stable tubulin-GTP cap at its two distinct ends. The gain and loss of GTP cap originate a phenomenon called dynamic instability which is crucial in various brain cells functions particularly in cognition and memory [32,33]. OST may stabilize MTs by conformational changes inducing tubulin to cause more stable tubulin-GTP caps. However, the results suggested that OST has not negatively affected MT dynamic instability and the depolymerization process. It is also possible that OST might reduce the rate of GTP hydrolysis or Pi release, preventing the tubulin-GTP cap loss in order to stabilize MT polymers.

Since Paclitaxel is an effective antimitotic agent binding to tubulin to suppress the MT catastrophic phase and to enhance nucleation and elongation phases of MT polymerization [34], the effect of Paclitaxel and OST along with their combinational effect on the tubulin assembly were compared. OST could enhance the MT polymerization process similar to paclitaxel; however, the evidence showed that OST has not disrupted MT dynamic instability and increased elongation rate significantly in contrast to Paclitaxel. It is indicated that the effect of paclitaxel on MT dynamic could not be inhibited in the presence of OST; however, the active sites for OST and paclitaxel might be the same or vicinal. Turbidimetry assay revealed that tubulin assembly increased in the presence of OST alone in comparison with the combination of paclitaxel and the same concentration of OST. Therefore, it seems that OST and paclitaxel may compete for binding to tubulin. On a whole, it was shown that OST indicated more efficacy rather than the combination of OST with Paclitaxel for enhancement of MT polymerization rate.

Colchicine is a natural toxic product and an effective inhibitor of tubulin assembly in vitro. Colchicine binds to tubulin to form a tight complex and induces tubulin conformational change and the tubulin-colchicine complex inhibits MT growth [35]. Controversial to our expectation, we observed that colchicine, which usually has a devastating impact on the MT polymer, could improve the stability of MTs when it was applied in the presence of OST. It seems that OST caused conformational changes in MT

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structure that may interfere in the colchicine binding site and inhibit the action of colchicine; however, it is clear-cut that colchicine has attenuated the stabilizing effect of OST on tubulin assembly.

The fluorescence intensity emission of tubulin was identified in order to investigate tubulin conformational changes in the presence of increasing concentrations of OST. ANS is a fluorescence probe utilized to study the surface hydrophobicity of the protein after interacting with a ligand. Therefore, the protein-ANS complex provides beneficial data about the possible alterations of the protein conformation [36]. The obtained outcome demonstrated that the complex of OST-tubulin affected the tubulin conformation due to the increment of ANS fluorescence emission of tubulin upon the addition of OST. In other words, OST could promote the exposure of tubulin hydrophobic regions, which have buried in the interior structure of the native tubulin, on the protein surface. The conformational changes of tubulin induced by OST binding could be an important factor for the enhancement of tubulin assembly.

In conclusion, although OST could stabilize MT polymers, it had no suppressive effects on their natural dynamic instability. We conclude that OST is a unique potent compound that could interact with tubulin in order to induce MT polymerization. Further studies are required to define OST efficacy for the treatment of neurodegenerative diseases.

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Appendixes

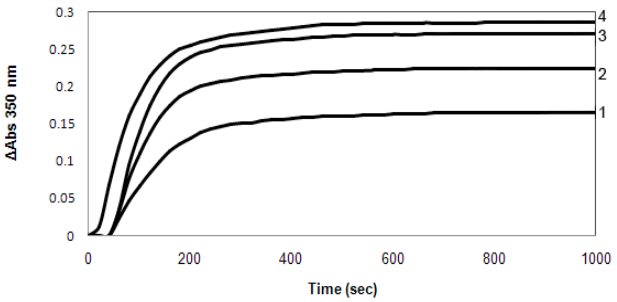


Figure 1. Effect of OST on MT Polymerization in vitro

Note: Tubulin (2 mg/ml) was incubated at 37 ℃ in the presence of 1 mM GTP, different concentrations of OST, and the same volume of solvent as a control. MT polymerization was monitored by measuring turbidity every 20 seconds at 350 nm. 1) 0 μM OST (Control); 2) 2 μM OST; 3) 5 μM OST; 4) 10 μM OST. The experiment was repeated four times.

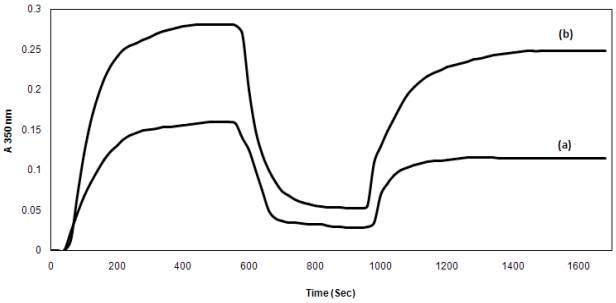
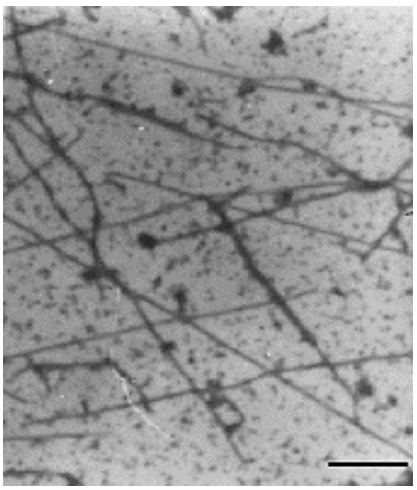
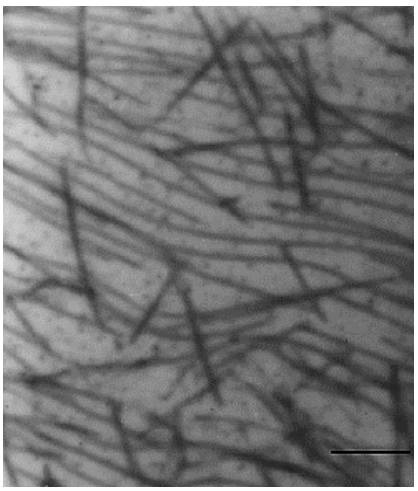


Figure 2. MT Repolymerization Assay

Note: Polymerized MT polymer (2 mg/ml) was disassembled by cooling down to 4 °C and then re-warming 37 ℃ to induce reassembly without any further addition of GTP or OST. Repolymerization was monitored by measuring the absorbance value every 20 seconds at 350 nm. a) 0 μM OST (Control); b) 10 μM OST.



(a)



(b)

Figure 3. Electron Microscopy Micrographs of MT Proteins

Note: MT proteins incubated with and without OST (10 μM) were negatively stained with 1% uranil acetate. a) MT polymers without OST; b) OST-treated MT polymers after 25 min of polymerization initiation. (Scale bars = 0.5 micrometer).

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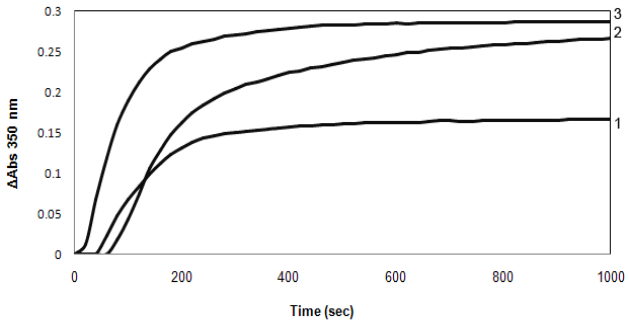

Figure 4. Tubulin Assembly Induced by OST and Paclitaxel

Note: Tubilin proteins (2 mg/ml) were incubated with OST, mixture of OST and Paclitaxel and equal volume of solvent as a control. Turbidity changes were observed every 20 seconds at 350 nm. 1) 0 μM OST and Paclitaxel (Control); 2) 10 μM OST and 0.1 mM Paclitaxel; 3) 10 μM OST.

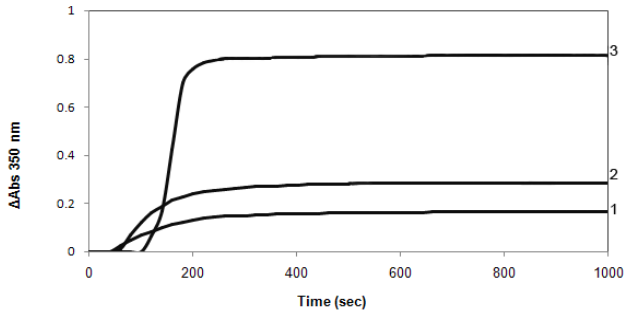

Figure 5. Tubulin Assembly Induced by OST and Colchicines

Note: Tubulin proteins (2 mg/ml) were incubated with OST, mixture of OST and Colchicine, and equal volume of solvent as a control. Turbidity changes were monitored every 20 second at 350 nm. 1) 0 μM OST and Colchicine (Control); 2) 10 μM OST and 0.1 mM Colchicine; 3) 10 μM OST.

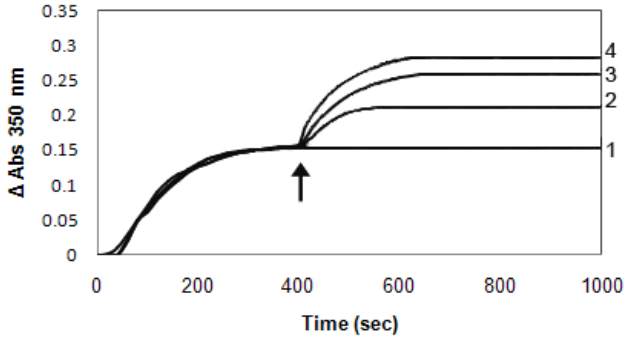

Figure 6. Effect of OST on MT Polymers at the Steady State Phase

Note: Tubulin proteins (2 mg/ml) were polymerized in the absence of OST. After 400 seconds from assembly initiation, various ranges of OST were added to the assembled tubulin in the steady state phase (displayed by the arrow). Polymerization process was recorded by turbidity changes at 350 nm. 1) 0 μM OST (Control); 2) 2 μM OST; 3) 5 μM OST; 4) 10 μM OST.

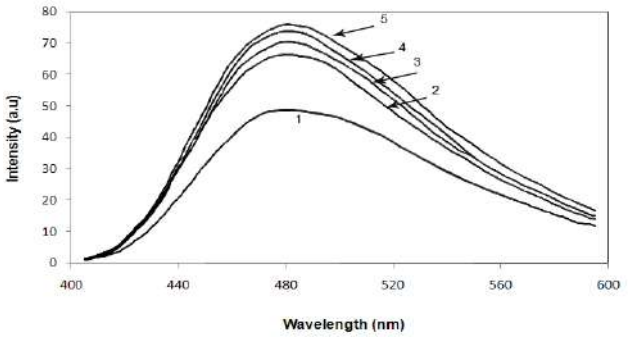

Figure 7. ANS Fluorescence Emission of Tubulin

Note: Tubulin proteins (2 μM) were incubated with various concentrations of OST (0, 2, 5, 7, 10 μM) for 10 min at 4 ℃. ANS (50 µM final concentration) was added and after 10 min incubation, fluorescence emissions were recorded from 400 to 600 nm following excitation at 380 nm. 1) 0 μM OST; 2) 2 μM OST; 3) 5 μM OST; 4) 7 μM OST; 5) 10 μM OST.

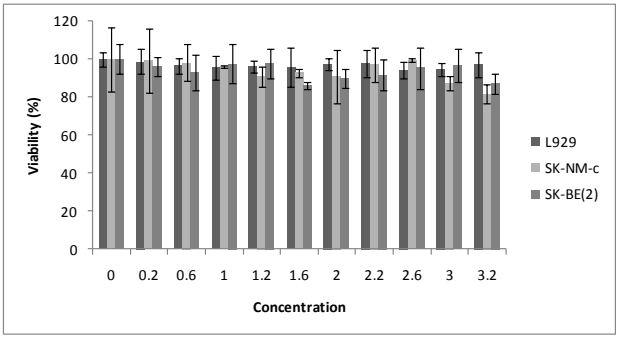

Figure 8. Viability Results of the Cell Culture Assesments

Note: SK-N-MC, SK-N-BE (2) and L929 Cells were treated with different concentrations OST ranged from 0.2 to 3.2 µM for 24 h and their viability was evaluated using MTT colorimetric assay.

References

[1] Castola, V., A. Bighelli, J. Casanova. Intraspecific chemical variability of the essential oil of Pistacia lentiscus L. from Corsica. Biochemical Systematics and Ecology, 2000, 28(1): 79-88.

[2] Fleisher, Z., A. Fleisher. Volatiles of the Mastic Tree—Pistacia lentiscus L. Aromatic Plants of the Holy Land and the Sinai. Part X. Journal of Essential Oil Research, 1992, 4(6): 663-665.

[3] Magiatis, P., et al.. Chemical composition and antimicrobial activity of the essential oils of Pistacia lentiscus var. chia. Planta Med, 1999, 65(8): 749-52.

[4] Miyamoto, T., T. Okimoto, M. Kuwano. Chemical Composition of the Essential Oil of Mastic Gum and their Antibacterial Activity Against Drug-Resistant Helicobacter pylori. Nat Prod Bioprospect, 2014, 4(4): 227-31.

[5] Koutsoudaki, C., M. Krsek, A. Rodger. Chemical composition and antibacterial activity of the essential

 DOI: https://doi.org/10.30564/jhp.v1i2.1589

oil and the gum of Pistacia lentiscus Var. chia. J Agric Food Chem, 2005, 53(20): 7681-5.

[6] Al-Said, M.S., et al.. Evaluation of mastic, a crude drug obtained from Pistacia lentiscus for gastric and duodenal anti-ulcer activity. J Ethnopharmacol, 1986, 15(3): 271-8.

[7] Yaman-Sozbir, S., S. Ayaz-Alkaya, B. Bayrak-Kahraman. Effect of chewing gum on stress, anxiety, depression, self-focused attention, and academic success: A randomized controlled study. Stress Health, 2019. 35(4): 441-446.

[8] Ammari, M., et al.. Pistacia lentiscus oil attenuates memory dysfunction and decreases levels of biomarkers of oxidative stress induced by lipopolysaccharide in rats. Brain Res Bull, 2018, 140: 140-147.

[9] Saidi, S.A., et al.. Liver injury following small intestinal ischemia reperfusion in rats is attenuated by Pistacia lentiscus oil: antioxidant and anti-inflammatory effects. Arch Physiol Biochem, 2017, 123(4): 199-205.

[10] Loizou, S., et al.. Chios mastic gum extract and isolated phytosterol tirucallol exhibit anti-inflammatory activity in human aortic endothelial cells. Exp Biol Med (Maywood), 2009, 234(5): 553-61.

[11] Nelson, R.. Microtubule-stabilising drugs may be therapeutic in AD. Lancet Neurol, 2005, 4(2): 83-4.

[12] Cornet, B., et al.. Refined three-dimensional solution structure of insect defensin A. Structure, 1995. 3(5): 435-48.

[13] Downing, K.H., E. Nogales. Tubulin and microtubule structure. Curr Opin Cell Biol, 1998, 10(1): 16-22.

[14] Nogales, E.. Structural insights into microtubule function. Annu Rev Biochem, 2000, 69: 277-302.

[15] Park, J.H., A. Roll-Mecak. The tubulin code in neuronal polarity. Curr Opin Neurobiol, 2018, 51: 95-102.

[16] Conde, C., A. Caceres. Microtubule assembly, organization and dynamics in axons and dendrites. Nat Rev Neurosci, 2009, 10(5): 319-32.

[17] Downing, K.H.. Structural basis for the interaction of tubulin with proteins and drugs that affect microtubule dynamics. Annu Rev Cell Dev Biol, 2000, 16: 89-111.

[18] Qian, A., P.R. Burton, R.H. Himes, A comparison of microtubule assembly in brain extracts from young and old rats. Brain Res Mol Brain Res, 1993, 18(1-2): 100-6.

[19] Nakayama, T., T. Sawada, Involvement of microtubule integrity in memory impairment caused by colchicine. Pharmacol Biochem Behav, 2002, 71(1-2): 119-38.

[20] Trojanowski, J.Q., et al.. Microtubule-stabilising drugs for therapy of Alzheimer's disease and other neurodegenerative disorders with axonal transport impairments. Expert Opin Pharmacother, 2005, 6(5): 683-6.

[21] Ballatore, C., et al.. Microtubule stabilizing agents as potential treatment for Alzheimer's disease and related neurodegenerative tauopathies. J Med Chem, 2012, 55(21): 8979-96.

[22] Zarei Jaliani, H., et al.. The effect of the crocus sativus L. Carotenoid, crocin, on the polymerization of microtubules, in vitro. Iran J Basic Med Sci, 2013, 16(1): 101-7.

[23] Abidi, A., et al.. Protective Effect of Pistacia lentiscus Oil Against Bleomycin-Induced Lung Fibrosis and Oxidative Stress in Rat. Nutr Cancer, 2017, 69(3): 490-497.

[24] Miller, H.P., L. Wilson, Preparation of microtubule protein and purified tubulin from bovine brain by cycles of assembly and disassembly and phosphocellulose chromatography. Methods Cell Biol, 2010, 95: 3-15.

[25] Weingarten, M.D., et al.. Properties of the depolymerization products of microtubules from mammalian brain. Biochemistry, 1974, 13(27): 5529-37.

[26] Carlsson, N., et al.. Quantification of protein concentration by the Bradford method in the presence of pharmaceutical polymers. Anal Biochem, 2011, 411(1): 116-21.

[27] Scudiero, D.A., et al.. Evaluation of a soluble tetrazolium/formazan assay for cell growth and drug sensitivity in culture using human and other tumor cell lines. Cancer Res, 1988, 48(17): 4827-33.

[28] Solomaha, E., H.C. Palfrey, Conformational changes in dynamin on GTP binding and oligomerization reported by intrinsic and extrinsic fluorescence. Biochem J, 2005, 391(Pt 3): 601-11.

[29] Penazzi, L., L. Bakota, R. Brandt, Microtubule Dynamics in Neuronal Development, Plasticity, and Neurodegeneration. Int Rev Cell Mol Biol, 2016, 321: 89-169.

[30] Brandt, R., L. Bakota, Microtubule dynamics and the neurodegenerative triad of Alzheimer's disease: The hidden connection. J Neurochem, 2017, 143(4): 409-417.

[31] Moeini, R., et al.. Pistacia Genus as a Potential Source of Neuroprotective Natural Products. Planta Med, 2019, 85(17): 1326-1350.

[32] Manka, S.W., C.A. Moores. The role of tubulin-tubulin lattice contacts in the mechanism of microtubule dynamic instability. Nat Struct Mol Biol, 2018, 25(7): 607-615.

[33] Atarod, D., et al.. Microtubule Dynamicity Is More

DOI: https://doi.org/10.30564/jhp.v1i2.1589

Important than Stability in Memory Formation: an In Vivo Study. J Mol Neurosci, 2015, 56(2): 313-9.

[34] Alves, R.C., et al.. Characteristics, Properties and Analytical Methods of Paclitaxel: A Review. Crit Rev Anal Chem, 2018, 48(2): 110-118.

[35] Dasgeb, B., et al.. Colchicine: an ancient drug with novel applications. Br J Dermatol, 2018, 178(2): 350-356.

[36] Dadras, A., et al.. In vitro study on the alterations of brain tubulin structure and assembly affected by magnetite nanoparticles. J Biol Inorg Chem, 2013, 18(3): 357-69.

 DOI: https://doi.org/10.30564/jhp.v1i2.1589

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ARTICLE

In Silico Study Predicts CCDC69 as a Novel Tumour Suppressor Gene in HER2+ Breast Cancer

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ABSTRACT

Differential gene expression analysis using databases followed by overall survival (OS) analysis is currently used to identify different oncogenes and tumour suppressor genes. The present study identified coiled coiled domain containing protein 69 (CCDC69) as a tumour suppressor gene in breast cancer by differential gene expression analysis using TCGA dataset for breast adenocarcinoma (BRCA) followed by OS and relapse free survival (RFS) analysis using Kaplan Meier (KM) plotter tool. CCDC69 was observed to be down regulated in tumour of breast cancer patients in BRCA. Following OS analysis for different breast cancer sub-types, low expression of CCDC69 has been observed to be associated with poor survival in HER2+ breast cancer only. CCDC69 was also found to be down regulated in different HER2+ breast cancer cells by analysing Gene Expression Omnibus (GEO) database. Additionally, CCDC69 was found to be under expressed in single cell HER2 positive population, which is evident from the single cell expression ATLAS database. Furthermore, CCDC69 has been observed to be lowly expressed with overexpression of HER2 in breast cancer by co-expression study. The possible mechanism of CCDC69 down regulation in HER2+ breast cancer was resolved using P-SCAN tool. P-SCAN analysis suggested a group of transcription factors (TFs) among which androgen receptor (AR) has been selected as the probable TF that could play a role in CCDC69 down regulation in HER2+ breast cancer. Moreover, overexpression of AR has been observed in BRCA and HER2+ single cell population. AR has also been observed to be co-expressed positively with HER2, but negatively with CCDC69 in breast cancer. Down regulation of CCDC69 can be predicted to stabilize microtubule formation following stimulation of cell growth and cell migration leading to HER2+ breast cancer progression and metastasis.

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Abbreviation

CCDC69: Coiled Coiled Domain Containing protein 69; TCGA: The Cancer Genome Atlas; BRCA: Breast Adenocarcinoma; GEPIA: Gene Expression Profiling Interactive Analysis; MTCI: Molecular Therapeutics for Cancer, Ireland; GEO: Gene Expression Omnibus; OS: Oveall Survival; RFS: Relapse Free Survival

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1. Introduction

Breast cancer is a rapidly growing and the most predominant disease of cancer mediated morbidity and mortality in women all over the world. The possibility of recurrence of breast cancer depends on the tumour size, grade, nodal status, tumour subtype and especially mode of therapy [1]. The presence of hormonal receptors "estrogen receptor (ER) and progesterone receptor (PR)" and HER-2 "human epidermal growth factor receptor 2" receptor in the tumour cells have been selected as one of the important factors for the diagnosis of breast cancer. Based on the expression of these receptors, breast cancer has been divided into different sub-types: luminal A, luminal B, triple negative and HER2 positive [2]. Over the past decade, advancement with cancer research using different databases, high throughput technologies, clinical trial allowed the discovery of new drugs for treatment of breast cancer. Better understanding of phenotype and heterogeneity of breast cancer has opened the possibility for the development of more effective and individualized approach to treatment. The most common treatments that currently available are surgery, radiation therapy and chemotherapy. Surgery and radiation therapy are the most common treatment options for primary tumours and large metastases, while chemotherapy is the best treatment option for metastatic tumours. Traditional anticancer chemotherapeutic drugs inhibit cancer cell division and DNA replication leading to prevention of breast cancer. However, they do not seem to be appropriate in all types of cancer. Regarding the background and disadvantage of chemotherapy, complementary treatment modalities are being widely explored in recent years. For example, molecular therapy, anti-angiogenesis therapy, immunotherapy, apoptosis regulation, signal-transduction therapy, targeted radionuclide therapy and nucleic acid based therapies have attracted attention from the health care system [3]. Therefore, finding new therapeutic targets such as oncogenes, tumour suppressor genes, signalling molecules are the recent trends of research for targeted therapy to combat cancer. Targeted therapy exerts its anticancer effects through multiple mechanisms, including arrest of cell growth, induction of apoptosis, and suppression of spreading of tumour (a.k.a tumour metastasis) as well as immune function regulation and multidrug resistance reversal.

Differential gene expression analysis using different databases followed by OS is important in the cancer research. This can help in identifying different oncogenes and tumour suppressor genes. The aim of our study was to identify a novel tumour suppressor gene in breast cancer. Herein, we have identified CCDC69 as a tumour suppressor gene in breast cancer by differential gene expression analysis using TCGA dataset for BRCA followed by OS and RFS analysis using KM plot and CCDC69 has been found to be down regulated in HER2+ breast cancer. Transcription factor, AR was predicted by our *in silico* study as the key regulator for the down regulation of CCDC69 in HER2+ breast cancer.

2. Methods and Tools

2.1 Differential Gene Expression Analysis Using Gene Expression Profiling Interactive Analysis (GEPIA) Tool

GEPIA allows users to input custom statistical methods and thresholds for TCGA dataset to dynamically obtain differentially expressed genes. The differential gene expression analysis for the present study has been done using GEPIA online server tool. Details of the method are described in the GEPIA website [4].

2.1 Overall Survival (OS) Analysis Using Kaplan Meier Plotter

The Kaplan Meier plotter is capable to assess the effect of 54k genes on survival in 21 cancer types. The largest datasets include breast (n=6,234), ovarian (n=2,190), lung (n=3,452), and gastric (n=1,440) cancer. Details of the method are described in the KM plotter website [5].

2.3 MTCI Breast Cancer Overall Survival Tool

Molecular Therapeutics for Cancer, Ireland (MTCI) is a Science Foundation Ireland-funded strategic research cluster which aims to discover and develop new anti-cancer drugs. Details of the method are described in the MTCI website.

2.4 Single Cell Expression Atlas - EMBL-EBI

EMBL-EBI Single Cell Expression Atlas, an open public repository of single cell gene expression data. Details of the method are described in the Single Cell Expression Atlas - EMBL-EBI website.

2.5 Statistical Analysis

Statistical analyses were followed according to the analysis has been done in different tools that are used in the present study. Details of the method are available in the website of the tools.

3. Results and Discussion

3.1 Differential Gene Expression Analysis Identifies CCDC69 as a Tumour Suppressor Gene

To find out a novel tumour suppressor gene in breast

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cancer, we have first undertaken differential gene expression analysis (log2 fold change cut off and p value were considered ≤-2 and ≤.001 respectively) for BRCA in TCGA dataset using GEPIA (Gene Expression Profiling Interactive Analysis) tool followed by OS and RFS analysis using KM-Plotter. We have selected CCDC69, which may act as a tumour suppressor in breast cancer following literature study since we did not find significant number of publications that suggests CCDC69 role as tumour suppressor in breast cancer. CCDC69 has been observed to be down regulated in different types of cancer including breast in TCGA dataset (Figure 1 A-C). Especially, CCDC69 mRNA was under expressed in breast tumour sample (~6 fold compared to normal) in BRCA in TCGA dataset (Figure 1D). OS analysis and RFS analysis for breast cancer using KM-Plot suggested low expression of CCDC69 is associated with poor survival with a p value of $8.2e^{-07}$ and $7.8e^{-16}$, respectively, which predicts that CCDC69 may strongly acts as a tumour suppressor gene in breast cancer (Figure 2A).

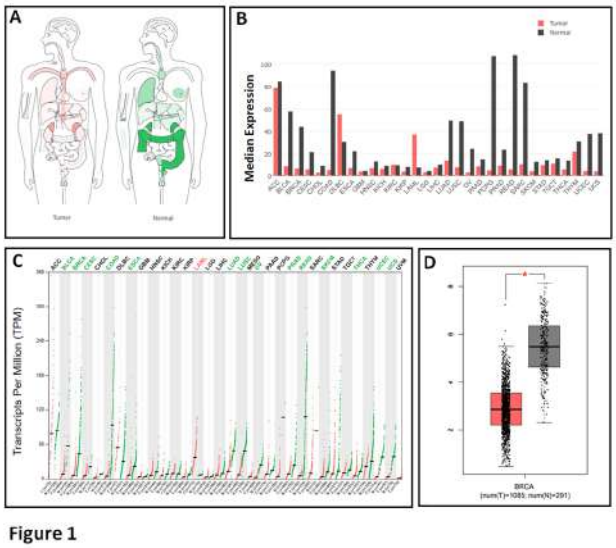

Figure 1

Figure 1. CCDC69 expression in different cancers

3.2 OS Analysis for Different Breast Cancer Subtypes Predicts that CCDC69 may Play Role only in HER2+ Breast Cancer

Although low expression of CCDC69 expression was found to be associated with poor survival in breast cancer, it is important to know in which breast cancer sub-type CCDC69 may play role as a tumour suppressor. To this end, OS analysis has been performed for different breast cancer subtypes using MTCI breast cancer survival analysis tool. CCDC69 expression was not found to be associ-

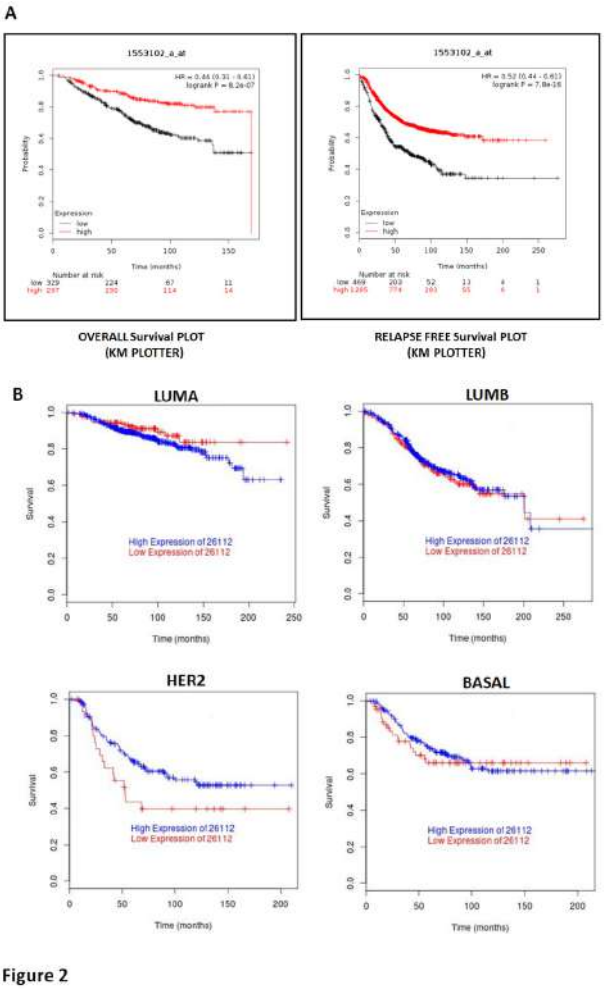

Figure 2

Figure 2. A) Overall survival analysis plot of CCDC69 in breast cancer; B) Overall survival analysis plot of CCDC69 in different breast cancer sub-types

ated with poor survival in LUMA, LUMB and triple negative breast cancer subtypes (p values are 0.216, 0.656 and 0.677 respectively); however, low expression of CCDC69 was found to be associated with poor survival in HER2+ breast cancer population with a p value of 0.05 (Figure 2B). Next to determine whether CCDC69 is under expressed in HER2+ breast cancer cell lines, we used GEO dataset to see CCDC69 mRNA expression in different breast cancer cells. Our study revealed that CCDC69 is down regulated in different HER2+ positive breast cancer cell lines (Figure 3A). We have also checked CCDC69 expression in single cell population isolated from breast cancer patients using single cell expression ATLAS database. Interestingly, CCDC69 was found very lowly expressed in HER2+ single cell population (Figure 3B). Additionally, we determined whether CCDC69 is co-expressed with HER2 in breast cancer by co-expression analysis and the

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study indicated that CCDC69 is co-expressed negatively with HER2 (Figure 3C). Overall, the above observations strongly support CCDC69 down regulation in HER2+ breast cancer cells.

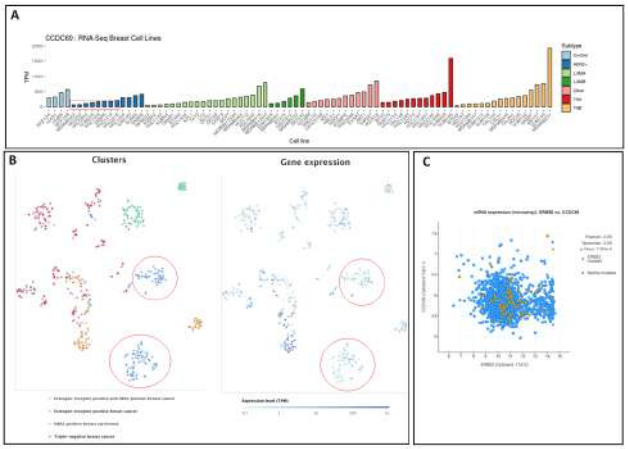

Figure 3. CCDC69 expression in different breast cancer cell lines and single cell population

3.3 Role of CCDC69 Co-expressed Genes in Breast Cancer

Gene co-expression analysis is used to identify the function of an unknown gene in biological processes to prioritize the role of the candidate gene in diseases and to determine transcriptional regulatory programmes [6]. To gain an insight in the function of CCDC69 in biological process or in disease regulation, we did co-expression study with the use of genevestigator tool. Co-expression analysis revealed 25 genes which are further analysed for their role in breast cancer using TCGA dataset for BRCA (Figure 4A). Unfortunately, expression of not a single gene among the 25 genes was found to be changed significantly in tumour tissue compared to normal tissue in TCGA dataset (Figure 4B). Therefore, it may be concluded that CCDC69 is down regulated exclusively in BRCA in comparison to its co-expressed genes. Next to determine the function of CCDC69 or its role in signalling pathways, we have used gene analytics tool. No signalling pathways, however, has been observed where CCDC69 appears to be involved and could play a significant role leading to breast cancer progression. Nevertheless, literature study indicated a role of CCDC69 in the activation of p14ARF/MDM2/p53 signalling pathway in ovarian cancer [7]. Thus, in vitro study that shows a functional role of CCDC69 in breast cancer will be required in future.

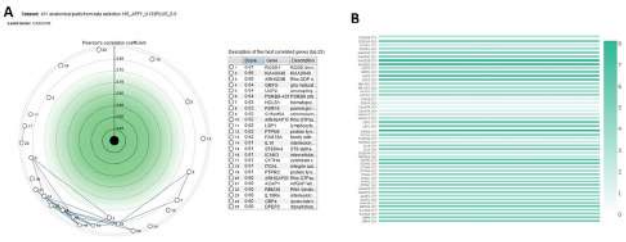

Figure 4. CCDC69 co expressed genes and their role in breast cancer

3.4 AR may be Involved in the down Regulation of CCDC69 in HER2+ Breast Cancer

Since CCDC69 was found to be down regulated in HER2+ breast cancer and as OS analysis strongly supports its role as a tumour suppressor; therefore it appears essential to know the mechanism by which CCDC69 is down regulated. To this end, P-SCAN analysis was performed to identify possible transcription repressors that may bind to the CCDC69 promoter region leading to suppression of CCDC69 mRNA transcription. P-SCAN analysis identified top 15 transcription factors with significant p value, of which androgen receptor (AR) was selected as the probable regulator of CCDC69 (Figure 5A). AR was selected to cause CCDC69 mRNA down regulation in HER2+ breast cancer because AR was found to be co-expressed with HER2 positively; however, negatively with CCDC69 by co-expression analysis (Figure 5B & C). Additionally, AR was found to be overexpressed in the tumour of breast cancer patients TCGA dataset for BRCA (Figure 5D). Interestingly, gene expression analysis for AR in single cell dataset suggests that AR is overexpressed in HER2+ breast cancer subtype (Figure 5E).

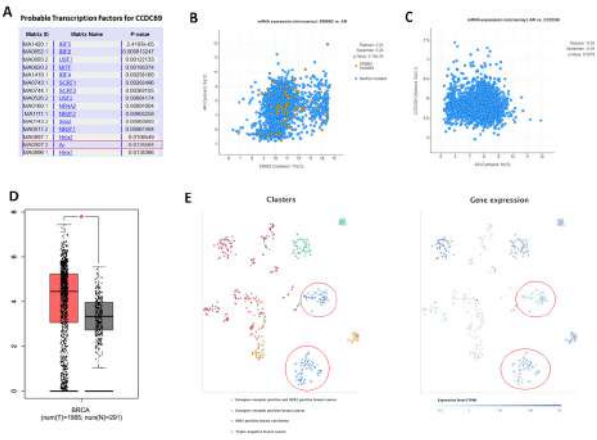

Figure 5. Mechanism of CCDC69 down regulation in HER2+ breast cancer

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Transcription factors can act both as a transcriptional activator or repressor depending on the cell system. AR has been indicated to act as a transcriptional repressor in a recently published article [8]. Therefore, it is suggested that overexpressed AR may bind to the CCDC69 promoter leading to the transcriptional repression of CCDC69 in HER2+ breast cancer.

4. Conclusion and Future Prospects

The present in silico study identifies CCDC69 as a novel tumour suppressor gene in HER2+ breast cancer. The probable mechanism for CCDC69 down regulation in HER2+ breast cancer was resolved and AR is suggested as the predominant transcriptional repressor for the under expression of CCDC69 mRNA in HER2+ breast cancer. The present study will open the possibility of a wet lab work to verify the role of CCDC69 as a tumour suppressor in HER2+ breast cancer. The present study also suggests that AR can be a therapeutic target in HER2+ breast cancer and its inhibition can be considered as a therapeutic strategy in HER2+ breast cancer treatment. Functional study of CCDC69 and identifying the mechanism by which CCDC69 play a role in HER2+ breast cancer are also seem to be promising for future study. Recent study demonstrated that CCDC69 can destabilize microtubules [9]. Knockdown of CCDC69 by RNAi leads to the formation of aberrant central spindles and interferes with the localization of midzone components such as aurora B, PRC1, MgcRacGAP, and Plk1 leading to stabilization of microtubules [9]. It is well known that microtubules stabilization induces cell proliferation and migration [10,11]. Conceivably, it can be speculated that down regulation of CCDC69 may stabilize microtubule formation and subsequently stimulates cell growth and migration leading to breast cancer progression and metastasis. Hence, overexpression of CCDC69 in HER2+ breast cancer may be a therapeutic strategy for the treatment in future.

Conflict of Interest

There is no conflict of interest.

References

[1] McAnena, P.F., McGuire, A., Ramli, A., Curran, C., Malone, C., McLaughlin, R., Barry, K., Brown, J.A.L., Kerin, M.J., Breast cancer subtype discordance: impact on post-recurrence survival and potential treatment options, BMC Cancer, 2018, 18(18): 203.

[2] Edge, S.B., Compton, C.C., The American joint committee on cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM, 2010, Ann. Surg. Oncol, 2010, 17: 1471–1474.

[3] Ke, X., Shen, L., Molecular targeted therapy of cancer: The progress and future prospect, Frontiers in Laboratory Medicine, 2017, 1: 69-77.

[4] Tang, Z., Li, C., Kang, B., Gao, G., Li, C., Zhang, Z., GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses, Nucleic Acids Res., 2017, 45: 98-102.

[5] An online survival analysis tool to rapidly assess the effect of 22,277 genes on breast cancer prognosis using microarray data of 1,809 patients, Breast Cancer Res Treatment, 2010.

[6] Van, D.S., Vosa, U., Graaf, A., Franke, L., Magalhaes, J.P., Gene co-expression analysis for functional classification and gene-disease predictions, Brief Bioinform., 2018, 19: 575-592.

[7] Cui, L., Zhou, F., Chen, C., Wang, C.C., Overexpression of CCDC69 activates p14(ARF)/MDM2/p53 pathway and confers cisplatin sensitivity, J Ovarian Res., 2019, 12: 4.

[8] Clocchiatti, A., Ghosh, S., Procopio, M.G., Mazzeo, L., Bordignon, P., Ostano, P., Goruppi, S., Bottoni, G., Katarkar, A., Levesque, M., Kölblinger, P., Dummer, R., Neel, V., Özdemir, B.C., and Dotto, G.P., Androgen receptor functions as transcriptional repressor of cancer-associated fibroblast activation, J. Clin. Invest., 2018, 128: 5531-5548.

[9] Pal, D., Wu, D., Haruta, A., Matsumura, F., Wei, Q., Role of a novel coiled-coil domain-containing protein CCDC69 in regulating central spindle assembly, Cell Cycle., 2010, 9: 4117-4129.

[10] Fanale, D., Bronte, G., Passiglia, F., Calo, V., Castiglia, M., Piazza, D.F., Barraco, N., Cangemi, A., Catarella, M.T., Insalaco, L., Listì, A., Maragliano, R., Massihnia, D., Perez, A., Toia, F., Cicero, G., Bazan, V., Stabilizing versus destabilizing the microtubules: a double-edge sword for an effective cancer treatment option?, Anal. Cell. Pathol., 2015, 2015: 690916.

[11] Escuin, D., Kline, E.R., Giannakakou, P., Both microtubule-stabilizing and microtubule-destabilizing drugs inhibit hypoxia-inducible factor-1alpha accumulation and activity by disrupting microtubule function, Cancer. Res., 2005, 65: 9021-9028.

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- Program: Microsoft Word (preferred)
- Font: Times New Roman
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- Paragraph: Justified
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All articles should include a cover letter as a separate document.

The cover letter should include:

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Declaration

v Conflict of Interest

Examples of conflicts of interest include (but are not limited to):

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- Honoria
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This section confirms that written consent was obtained from all participants prior to the study.

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Eg. Name of Trial Registry: Trial Registration Number

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Supplementary figures, small tables, text etc.

As supplementary data/information is not copyedited/proofread, kindly ensure that the section is free from errors, and is presented clearly.

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A general introduction to the research topic of the paper should be provided, along with a brief summary of its main results and implications. Kindly ensure the abstract is self-contained and remains readable to a wider audience. The abstract should also be kept to a maximum of 200 words.

Authors should also include 5-8 keywords after the abstract, separated by a semi-colon, avoiding the words already used in the title of the article.

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Titles should be reflected as font size 26, and in bold type.

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The introduction should highlight the significance of the research conducted, in particular, in relation to current state of research in the field. A clear research objective should be conveyed within a single sentence.

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In this section, the results of the experiments conducted can be discussed in detail. Authors should discuss the direct and indirect implications of their findings, and also discuss if the results obtain reflect the current state of research in the field. Applications for the research should be discussed in this section. Suggestions for future research can also be discussed in this section.

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This section offers closure for the paper. An effective conclusion will need to sum up the principal findings of the papers, and its implications for further research.

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Ⅺ. Glossary of Publication Type

J = Journal/Magazine

M = Monograph/Book

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Conflicts of interest, acknowledgements, and publication ethics should also be declared in the final version of the manuscript. Instructions have been provided as its counterpart under Cover Letter.

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