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Storage of Medicines and Temperature Control at Community Pharmacies in Rural District of Sindh, Pakistan: An Exploratory Cross-Sectional Study page 17

From the Editor

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This issue of the journal has rich number of research papers in addition to review on self plagiarism issue and a CME article.

A joint paper from Australian and Iran looks at the background and history of plagiarism and self plagiarism, reviews aspects of academic self plagiarism from the academic, the institution and publisher's point of view and provides a handy check-list of the current definitions and requirements.

A paper from Pakistan attempt to estimate the proportion of pharmacies with high temperature (>25°C) inside pharmacy outlets in two talukas (sub-districts) of district Thatta, Sindh. The authors stressed that medicines are the essential tools for preventive, curative and control of diseases. If these medicines are ineffective then its aftermath can cause wastage of resources. Medicines lose their required effectiveness due to inadequate storage on required temperature. An exploratory cross sectional study design was conducted from August 2013 to August 2014. All pharmacies of two talukas were approached by doing a census. Descriptive analysis was done to calculate the frequencies and proportions. All pharmacies (n=62) were having a temperature of >25?C inside the pharmacies. Medicines were exposed to sunlight in 39 (63%) of the pharmacies and 39 (63%) of pharmacies had refrigerators to keep insulin and vaccines. Median duration of electricity shut downs was 12 hours per day and 11% of the pharmacies had back up power supply. The authors concluded that more than a quarter of pharmacy owners were aware about concluded that more than a quarter of pharmacy owners were aware about maintaining the required temperature of < 25◦C but none of them was maintaining required temperature. Considering the electricity shut down, it is important to make cost effective and long term strategies to maintain the efficacy of medicines. Proper legislation need to be enforced with continuing training programs for pharmacy owners. Further research is required to explore different ways of maintaining required temperature to ensure the adequate efficacy of medicines.

A paper from Turkey looked at Left renal atrophy in sickle cell diseases. The authors were intereted to understand whether or not there is a difference according to renal atrophy between the left and right sides in sickle cell diseases (SCDs). All patients with SCDs were enrolled into the study. The study included 311 patients (153 females). There were seven cases (2.2%) with left renal atrophy against one case (0.3%) with right renal atrophy ($p<0.001$). Associated thalassemias were detected in 44.0% and splenomegaly in 12.5% of the patients. There were digital clubbing in 6.4%, chronic obstructive pulmonary disease in 4.8%, leg ulcers in 12.8%, stroke in 7.0%, chronic renal disease in 8.6%, pulmonary hypertension in 11.8%, cirrhosis in 3.5%, coronary heart disease in 8.0%, and exitus in 5.7% of the patients. The authors concluded that renal atrophy is significantly higher on the left side in SCDs. Splenomegaly induced flow disorders in left renal vessels, structural anomalies of the left renal vein including nutcracker syndrome and passage behind the aorta, and possibly the higher arterial pressure of left kidney due to the shorter distance to heart as an underlying cause of endothelial damage induced atherosclerosis may be some of the possible causes. Because of the higher prevalences of left varicocele probably due to drainage of left testicular vein into the left renal vein, high prevalences of associated thalassemias with SCDs as a cause of splenomegaly, and tissue ischemia and infarctions induced edematous splenomegaly in early lives of the SCDs cases, splenomegaly induced flow disorders of left renal vein may be the most significant cause among them.

A paper from Kuwait looked at the effect of eye drop excipients against Acanthamoeba polyphaga. They screened a variety of such eye drop excipients used for bacterial keratitis in order to identify any candidates that show inhibitory activity against Acanthamoeba polyphaga, one of the protozoal species responsible for the Acanthamoeba Keratitis. Acanthamoeba keratitis is a serious eye infection which is notoriously difficult to treat successfully. The currently employed drugs have significant disadvantages in that they have to be administered at hourly intervals for extended periods of time. The AlamarBlueTM assay has been optimized for determination of selected eye drop excipients efficacy against potentially pathogenic strain, Acanthamoeba polyphaga. The most effective agents were found to be fusidic acid and framycetin sulfate, with a combination of the two providing a reduction in A. polyphaga metabolic activity of around 75%. The authors concluded that these eye drop excipients can serve as new sources for the discovery and development of much needed new antimicrobials for both Acanthamoeba keratitis and bacterial keratitis.

The author of a paper from Iran advises that for novice researchers within the health domain it would be absolutely essential to determine when they should start writing and publishing an article based on their recent research project. There are plenty of reasons which justify writing an article as soon as the necessary data are gathered and analyzed. The aim of the article is to discuss some of these most important rationales.

Another paper from Australia, in a continuing series, highlights the practical issues regarding modern day office surgery and provides practice tips along with some graphic examples of issues, particularly what to do when errors occur.

Left renal atrophy in sickle cell diseases

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Abstract

Background: **We tried to understand whether or not there is a difference in occurrence of renal atrophy between the left and right sides in sickle cell diseases (SCDs).**

Methods: **All patients with SCDs were enrolled into the study.**

Results: **The study included 311 patients (153 females). There were seven cases (2.2%) with left renal atrophy against one case (0.3%) with right renal atrophy (p<0.001). Associated thalassemias were detected in 44.0% and splenomegaly in 12.5% of the patients. There was digital clubbing in 6.4%, chronic obstructive pulmonary disease in 4.8%, leg ulcers in 12.8%, stroke in 7.0%, chronic renal disease in 8.6%, pulmonary hypertension in 11.8%, cirrhosis in 3.5%, coronary heart disease in 8.0%, and exitus in 5.7% of the patients.**

Conclusion: **Renal atrophy is significantly higher on the left side in SCDs. Splenomegaly induced flow disorders in left renal vessels, structural anomalies of the left renal vein including nutcracker syndrome and passage behind the aorta, and possibly the higher arterial pressure of left kidney due to the shorter distance to heart as an underlying cause of endothelial damage induced atherosclerosis, may be some of the possible causes. Because of the higher prevalences of left varicocele probably due to drainage of left testicular vein into the left renal vein, high prevalences of associated thalassemias with SCDs as a cause of splenomegaly, and tissue ischemia and infarctions induced edematous splenomegaly in early lives of the SCDs cases, splenomegaly induced flow disorders of left renal vein may be the most significant cause among them.**

Key words: **Sickle cell diseases, splenomegaly, left renal vein, left renal atrophy**

Introduction

Arterio- or atherosclerosis, but not venosclerosis, is an inflammatory process, probably developing secondary to the much higher arterial pressure induced chronic endothelial damage all over the body. It may be the main cause of aging induced end-organ failures in human beings (1,2). It is a systemic and irreversible process initiating at birth, and accelerated by many factors. The accelerating factors known for the moment are collected under the heading of metabolic syndrome. Some reversible components of the syndrome are overweight, hypertriglyceridemia, hyperbetalipoproteinemia, dyslipidemia, white coat hypertension, impaired fasting glucose, impaired glucose tolerance, and smoking for the development of terminal consequences such as obesity, diabetes mellitus (DM), hypertension (HT), coronary heart disease (CHD), chronic obstructive pulmonary disease (COPD), cirrhosis, chronic renal disease (CRD), peripheric artery disease (PAD), stroke, and other end-organ failures (3-8). Sickle cell diseases (SCDs) are a prototype of the accelerated atherosclerosis (9,10), by which we can observe terminal consequences of the metabolic syndrome very early in life. SCDs are caused by homozygous inheritance of the hemoglobin S (Hb S). Hb S causes erythrocytes to change their normal elastic structures to hard bodies. Actually, rigidity instead of shapes of the erythrocytes is the central pathology of the SCDs. The rigidity process is probably present in whole life, but exaggerated with stresses. The erythrocytes can take their normal elastic structures after normalization of the stresses, but after repeated attacks of rigidity, they become hard bodies, permanently. The rigid cells induced chronic endothelial damage causes tissue ischemia, infarctions, and end-organ failures even in the absence of obvious vascular occlusions due to the damaged and edematous endothelium. We tried to understand whether or not there is a difference according to the renal atrophy between the left and right sides in the SCDs patients.

Materials and Methods

The study was performed in the Hematology Service of the Mustafa Kemal University between March 2007 and May 2013. All patients with SCDs were enrolled into the study. SCDs are diagnosed by the hemoglobin electrophoresis performed via high performance liquid chromatography (HPLC) method. Their medical histories including smoking habit, regular alcohol consumption, leg ulcers, and stroke were learnt. Cases with a history of three pack-year were accepted as smokers and cases with a history of regular alcohol consumption with one drink a day for three years were accepted as alcoholics. A check up procedure including serum iron, total iron binding capacity, serum ferritin, serum creatinine value on three occasions, hepatic function tests, markers of hepatitis viruses A, B, and C and human immunodeficiency virus, an electrocardiogram, a Doppler echocardiogram, an abdominal ultrasonography, and a computed tomography of the brain were performed. Cases with acute painful crisis or any other inflammatory event were treated at first, and then the spirometric pulmonary function tests to diagnose COPD, the Doppler echocardiography to measure the systolic pressure of pulmonary artery, renal and hepatic function tests, and measurement of serum ferritin level were performed on the silent phase. Renal atrophies were detected ultrasonographically. The criterion for diagnosis of COPD is post-bronchodilator forced expiratory volume in 1 second/forced vital capacity of less than 70% (11). Systolic pressure of the pulmonary artery of 40 mmHg or higher during the silent phase is accepted as pulmonary hypertension (12). CRD is diagnosed with a permanently elevated serum creatinine level of 1.3 mg/dL or higher on the silent phase. Cases with renal transplantation were put into the CRD group. Cirrhosis is diagnosed with hepatic function tests, ultrasonographic findings, ascites, and histologic procedure in case of requirement. Digital clubbing is diagnosed with the ratio of distal phalangeal diameter to interphalangeal diameter of higher than 1.0 and with the presence of Schamroth's sign (13,14). Associated thalassemias are diagnosed by serum iron, total iron binding capacity, serum ferritin, and the hemoglobin electrophoresis performed via HPLC method. A stress electrocardiography was performed in cases with an abnormal electrocardiogram and/or angina pectoris. A coronary angiography was obtained just for the stress electrocardiography positive cases. So CHD was diagnosed either with the Doppler echocardiographic findings as the movement disorders of the cardiac walls or angiographically. Mann-Whitney U test, Independent-Samples t test, and comparison of proportions were used as the methods of statistical analyses.

Results

The study included 311 patients with the SCDs (153 females and 158 males). The mean ages of them were 28.2 ± 9.2 (8-59) versus 29.9 ± 9.6 (6-58) years in females and males, respectively ($p>0.05$). Interestingly, there were seven cases (2.2%) of the left renal atrophy against only one case (0.3%) of the right renal atrophy ($p<0.001$) among the study cases (Table 1). On the other hand, associated thalassemias were detected in 44.0%, splenomegaly in 12.5%, and autosplenectomy in 48.5% of the SCDs patients. Although smoking was observed in 7.0% of the patients, there was only one case (0.3%) with regular alcohol consumption. Additionally, there were digital clubbing in 6.4%, COPD in 4.8%, leg ulcers in 12.8%, stroke in 7.0%, CRD in 8.6%, pulmonary hypertension in 11.8%, cirrhosis in 3.5%, CHD in 8.0%, and exitus in 5.7% of the cases with the SCDs. Prevalence of mortality were similar in both genders (5.2% versus 6.3% in females and males, respectively, $p>0.05$), and mean ages of the mortal cases were 32.1 versus 29.1 years in females and males, respectively ($p>0.05$) (Table 2). On the other hand, five of the CRD cases were on hemodialysis, and one with right renal transplantation. Histologic procedure for the diagnosis of cirrhosis was not required in any case. Although antiHCV was positive in two of the cirrhotics, HCV RNA was detected as negative by polymerase chain reaction in both. The solitary case of regular alcohol consumption was not cirrhotic at the time of study.

Table 1: Sickle cell patients with associated disorders

Variables	Prevalence
Left renal atrophy	2.2% (7)
Right renal atrophy	0.3% (1) (p<0.001)*
Thalassemias	44.0% (137)
Splenomegaly	12.5% (39)
Autosplenectomy	48.5% (151)
Smoking	7.0% (22)
Regular alcohol consumption	0.3% (1)
Digital clubbing	6.4% (20)
Chronic obstructive pulmonary disease	4.8% (15)
Leg ulcers	12.8% (40)
Stroke	7.0% (22)
Chronic renal disease	8.6% (27)
Pulmonary hypertension	11.8% (37)
Cirrhosis	3.5% (11)
Coronary heart disease	8.0% (25)
Exitus	5.7% (18)

Table 2: Features of the mortal cases

Variables	Female cases	Male cases	p-value
Prevalence	5.2% (8)	6.3% (10)	ns*
Mean age (year)	32.1 ± 10.5 (19-45)	29.1 ± 9.6 (19-50)	ns

Discussion

Nephrons are the basic functional units of the kidneys located in the renal parenchyma, and each kidney contains about one million nephrons. Renal atrophy is characterized by shrinkage of kidneys due to loss of nephrons. Loss of nephrons also causes shrinkages of the renal arteries and veins, secondarily. Renal diseases, urinary tract obstructions, or acute or chronic pyelonephritis may cause renal atrophy. Reflux nephropathy is characterized by renal damage due to the backflow of urine, and it may also cause renal atrophy. Renal atrophy may also be caused by the obstruction of urinary tract due to an increased pressure on it, or compression of the intrarenal veins or arteries. Obstructive uropathy causes a higher urinary pressure within the kidneys causing damage to the nephrons. Although the various etiologies, probably renal ischemia is the most frequent cause of the renal atrophy. Probably the most common cause of renal ischemia is the systemic atherosclerosis, and CRD due to the systemic atherosclerosis is common in elderlies. Although the younger mean ages, we detected CRD in 8.6% of all cases in the present study, since the SCDs are an accelerated systemic atherosclerotic process.

SCDs are accelerated systemic atherosclerotic processes (9) initiating at birth, and by which we can observe final consequences of the systemic atherosclerosis which began 30 or 40 years earlier in life. Actually name of the syndrome should be 'Rigid Cell Induced Chronic Endothelial Dysfunction' instead of the SCDs or sickle cell anemia since we cannot observe the sickle cells in the peripheric blood samples of cases with additional thalassemias, easily. On the other hand, the rigidity of the erythrocytes is the main problem instead of their shapes or severity of anemia. The rigid cells induced chronic endothelial damage causes tissue ischemia, infarction, and end-organ failures even in the absence of obvious vascular occlusions on the chronic background of damaged and edematous endothelium all over the body. Even there were patients with severe vision or hearing loss among the present study cases. The digital clubbing and recurrent leg ulcers may also indicate the chronic tissue hypoxia in such patients. Due to the reversibility of digital clubbing and leg ulcers with the hydroxyurea treatment, the chronic endothelial damage is probably prominent at the microvascular level as in diabetic microangiopathies, and reversible to some extent. Although large arteries and arterioles are especially important for blood carriage, capillaries are more important for tissue oxygenation. So passage of the rigid cells through the endothelial cells

cause damage on the capillaries. Reversibility of the process may probably be more in early years of life but it gets an irreversible nature over time. Thus endothelial cells all over the body are edematous and swollen due to the destructive process as in splenomegaly seen in early years of life. But the ischemic process terminates with tissue fibrosis and shrinkage all over the body as in autosplenectomy. Even there were four cases with total teeth loss and one case with right ovarian atrophy among the study cases. The solitary case of right renal atrophy may also be explained by the mechanism. On the other hand, anemia probably is not the cause of the end-organ failures in the SCDs, since we cannot observe any shortened survival in the thalassemia minor cases although the presence of a moderate anemia. Although the mean survivals were 42 and 48 years for males and females for the SCDs in the literature (15), they were 29.1 and 32.1 years in males and females in the present study, respectively. The great differences between the survival may be secondary to the initiation of hydroxyurea in infancy in such countries (16).

The accelerated atherosclerotic process can also affect the renal arteries, and may lead to poor perfusion of the kidneys leading to reduced renal function and failure. The right renal artery is longer than the left because of the location of the aorta, since the aorta is found on the left side of the body. Additionally, the right renal artery is lower than the left because of the lower position of the right kidney. So the left kidney possibly has a relatively higher arterial pressure due to the shorter distance to heart as an underlying cause of endothelial damage induced atherosclerosis. But according to our opinion, the accelerated atherosclerotic process alone cannot explain the significantly higher prevalence of renal atrophy on the left side (2.2% versus 0.3%, $p<0.001$) in the present study. The left renal atrophy has also been reported in the literature (17). On the other hand, the very high prevalences of associated thalassemias (44.0%) and splenomegaly (12.5%) with the SCDs cases may be important for the explanation, since spleen and left kidney are closely related organs which may also be observed with the development of varicose veins from the left renal vein at the splenic hilus in cirrhotic cases. Any pressure on the left kidney as in splenomegaly cases may cause torsion of the renal vein, and prevents its drainage. We especially think about the drainage problems at the venous level due to the much higher arterial pressure that cannot be obstructed easily and the much higher prevalence of varicocele in the left side in males (18-20).

Varicocele is a dilatation of pampiniform venous plexus within the scrotum. It occurs in 15-20% of all males and 40% of infertile males, since researchers documented a recurrent pattern of low sperm count, poor motility, and predominance of abnormal sperm forms in varicocele cases (21,22). Varicoceles are much more common (nearly 80% to 90%) in the left side due to several anatomic factors including angle at which the left testicular vein enters the left renal vein, lack of effective antireflux valves at the juncture of left testicular vein and left renal vein, the nutcracker syndrome, and some other left renal vein anomalies such as passage behind the aorta. The nutcracker syndrome results mostly from the compression of the left renal vein between the abdominal aorta and superior mesenteric artery, although other variants exist (23). It may cause hematuria and left flank pain (24). Since the left gonad drains via the left renal vein, it can also result in left testicular pain in men or left lower quadrant pain in women (25). Nausea and vomiting may result due to compression of the splanchnic veins (25). An unusual manifestation of the nutcracker syndrome includes varicocele formation and varicose veins in the lower limbs (26). Another study has shown that the nutcracker syndrome is a frequent finding in varicocele patients (27), so it should be routinely searched in cases with left varicocele.

As a conclusion, the renal atrophy is significantly higher on the left side in the SCDs cases. Splenomegaly induced flow disorders in the left renal vessels, structural anomalies of the left renal vein including nutcracker syndrome and passage behind the aorta, and possibly the higher arterial pressure of the left kidney due to the shorter distance to heart as an underlying cause of endothelial damage induced atherosclerosis may be some of the possible causes. Because of the higher prevalences of left varicocele probably due to drainage of left testicular vein into the left renal vein, high prevalences of associated thalassemias with the SCDs as a cause of splenomegaly, and tissue ischemia and infarctions induced edematous splenomegaly in early lives of the SCDs cases, splenomegaly induced flow disorders of the left renal vein may be the most significant cause among them.

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The effect of eye drop excipients against Acanthamoeba polyphaga by AlamarBlue™ assay

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Abstract

Objective: Based on the reduction of alamarBlueTM, we have therefore screened a variety of such eye drop excipients used for bacterial keratitis in order to identify any candidates that show inhibitory activity against *Acanthamoeba polyphaga*, one of the protozoal species responsible for the *Acanthamoeba* Keratitis.

Subjects and Methods: *Acanthamoeba* keratitis is a serious eye infection which is notoriously difficult to treat successfully. The currently employed drugs have significant disadvantages in that they have to be administered at hourly intervals for extended periods of time. The AlamarBlueTM assay has been optimized for determination of selected eye drop excipients efficacy against potentially pathogenic strain, *Acanthamoeba polyphaga*.

Results: The most effective agents were found to be fusidic acid and framycetin sulfate, with a combination of the two providing a reduction in *A. polyphaga* metabolic activity of around 75%.

Conclusion: These eye drop excipients can serve as new sources for the discovery and development of much needed new antimicrobials for both *Acanthamoeba* keratitis and bacterial keratitis.

Key words: AlamarBlue; *Acanthamoeba* keratitis; *Acanthamoeba polyphaga*

Introduction

Acanthamoeba keratitis is a serious eye infection caused by *Acanthamoeba* species of protozoa. These protozoa are present in the majority of water bodies, including sea water, sewage, soil and tap water. Previously a relatively rare condition, prevalence of *Acanthamoeba* keratitis is increasing [1]. This is mainly caused by the growing use of contact lenses, with approximately 85% of infections occurring in contact lens users [2, 4]. *Acanthamoeba polyphaga* is one of the two main protozoa species responsible for the condition. This microorganism has two stages to its life cycle, a rapidly reproducing trophozoite phase followed by encystation to form a robust double-layered cyst that allows survival under harsh conditions such as the presence of toxic chemicals [5]. This cyst stage provides a significant hurdle in the treatment of *Acanthamoeba* keratitis, with most drugs having demonstrated limited activity against it [6].

Chlorhexidine and polyhexamethylene biguanide (PHMB) are currently the standard treatments for the condition, being active against both the trophozoite and cyst phases of the organism. Diamidines such as hexamidine are sometimes used in conjunction with these; however, their use alone should be avoided owing to the development of resistance [4, 7]. A further issue associated with these agents is the necessity to apply them at hourly intervals for extended periods of time. Novel and more effective drugs for treating *Acanthamoeba* keratitis are therefore highly sought after. Phosphocholines have shown some promise, with inhibitory activity against Acanthamoeba and other parasites being demonstrated in vitro and in animal tests [8-11). A number of potential targets for new treatments have been identified. These include various components of the cell membrane, mitochondria, and protein synthesis pathways [12]. Such targets present a wide variety of agents that could be screened for activity against *A. polyphaga*. A selection of possible drugs of interest is already used in eye drop form; however, their efficacies for specifically treating *Acanthamoeba* keratitis have not yet been investigated.

In the present study, we have screened eight components of commercially available eye drop solutions in order to identify any agents with the potential for treating the condition. To allow for rapid analysis of all excipients simultaneously, we employed an alamarBlueTM microplate assay that has been previously verified for use in analysing the response of *A. polyphaga* to inhibitory drugs [13].

Materials and Methods

Culture of *A. polyphaga*

A. polyphaga (strain 1501/18) was obtained from Culture Collection of Algae and Protozoa (Lincoln, London). The cells were cultured in medium supplemented with 20% mycological peptone, 0.9% maltose, and 1% penicillin, streptomycin, and amphotericin B (all Sigma-Aldrich, Pennsylvania, USA). They were incubated in 75 cm^2 flasks at room temperature, when cultures reached 90-95% confluence.

Determination of optimal seeding densities for *Acanthamoeba*

After harvesting, *A. polyphaga* were diluted in culture medium to give a stock solution containing 8.0×10^5 cells/ml. Different concentrations of cells were used for tests of varying length. For the 24 hour test, 100 υl aliquots of a solution of 8.0×10^4 cells/ml were added to the wells of a 96-well plate. The seeding densities of A. polyphaga that attained close to 100% alamarBlue reduction were determined in assays conducted for total periods of 24 and 96 hours.

AlamarBlue growth inhibition assay

A stock solution of A. polyphaga was prepared at 8.0×10^5 cells/ml in culture medium. Inhibition tests were carried out for two different time periods. For the 24 hour test, 100 υl aliquots of a solution of 8.0×10^4 cells/ml were added to the wells of a 96-well plate. For the 96 hour test, 100 υ l of a solution of 1.25×10^3 cells/ml were added to each well. Each test was carried out in triplicate, with the experiment carried out twice. The different eye drop excipients to be tested were added to the wells at the concentrations related to the compound solubility. Synergistic effects of the eye drop excipients were determined by using different combinations of the agents. The cultures were incubated at room temperature for the duration of the test period, after which 10 υl of alamarBlue reagent (Life Technologies, Renfrew, UK) was added to each well and the plates were incubated for a further 6 hours. The absorbance of the solutions was then measured at 570 nm and 600 nm using Gemini EM Microplate Reader (Molecular Devices, Sunnyvale, USA). The percentage reduction of alamarBlue was then calculated according to the following equation:

{[(εox) λ2Aλ1] - [(εred) λ1 Aλ2 of treated Acanthamoeba]
/ [(εox) λ2A^0λ1]
- [(εred) λ1 A0λ2 of untreated Acanthamoeba]} X 100

Where εred λ1 is 155,677 (molar extinction coefficient of reduced alamarBlue™ at 570 nm); εred λ2 is 14,652 (molar extinction coefficient of reduced alamarBlue™ at 600 nm); εoxλ1 is 80,586 (molar extinction coefficient of oxidised alamarBlue™ at 570nm); εoxλ2 is 117,216 (molar extinction coefficient of oxidised alamarBlue™ at 600nm); A λ_1 is the absorbance of treated wells at 570nm; A λ_2 is the absorbance of treated wells at 600nm; A^0 λ_1 is the absorbance of cells with medium only as control wells at 570nm; A^0 λ_2 is the absorbance of untreated control wells at 600nm. These absorbance values were multiplied by 100 to give percentage of alamarBlue™ reduction comparison to untreated trophozoites cultured. The results were expressed as a mean for each triplicate ± the standard error (SE) (AbD Serotec, alamarBlue™ Assay).

Statistics

All tests were carried out twice in triplicate. As the results of the two experiments were highly similar, statistical analysis was carried out on the data from one experiment. Values are expressed as the mean with the standard

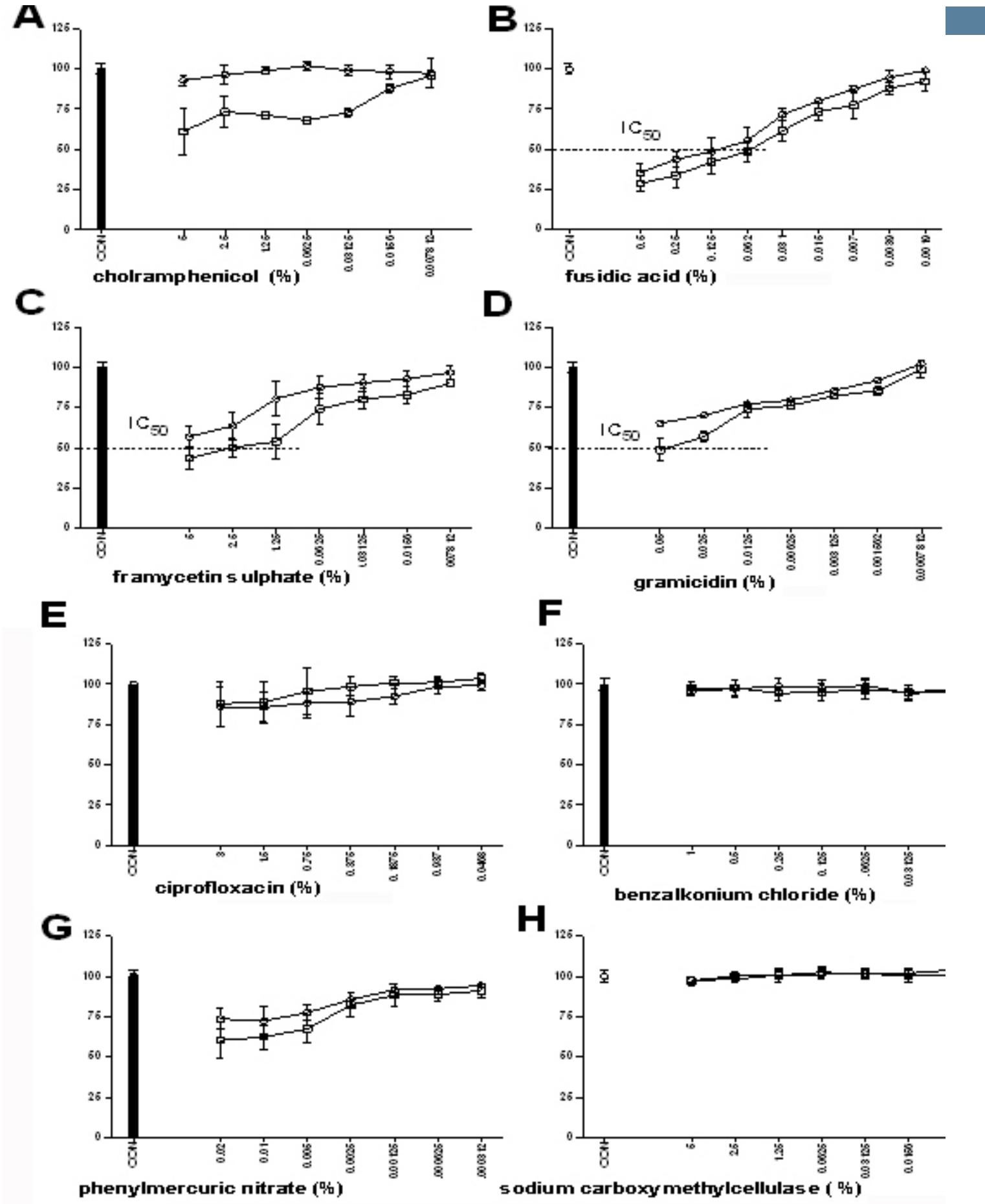

Figure 1: Relative susceptibilities of A. polyphaga (open circles 24 h) and (open squares 96 h) to the following: (A) cholamphenicol (no IC50 indicated), (B) fusidic acid (IC50 at 0.125 % for 24 h and 0.062 % for 96 h), (C) framycetin sulphate (IC50s at 2.5% for 96 h), (D) gramicidin (IC50 at 0.05 for 96 h), (E) ciprofloxacin (no IC50 indicated), (F) benzalkonium cholride (no IC50 indicated), (G) phenylmercuric nitrate (no IC50 indicated) and (H) sodium carboxymethylcellulase (no IC50 indicated). A. polyphaga to each of the eye drop excipients tested ($P < 0.05$). Acanthamoeba cell numbers were assessed by measuring the percent alamarBlue reduction relative to that for the untreated control cultures [CON] over 24 and 96 h. The results are expressed as the means for triplicate cultures ±SEs.

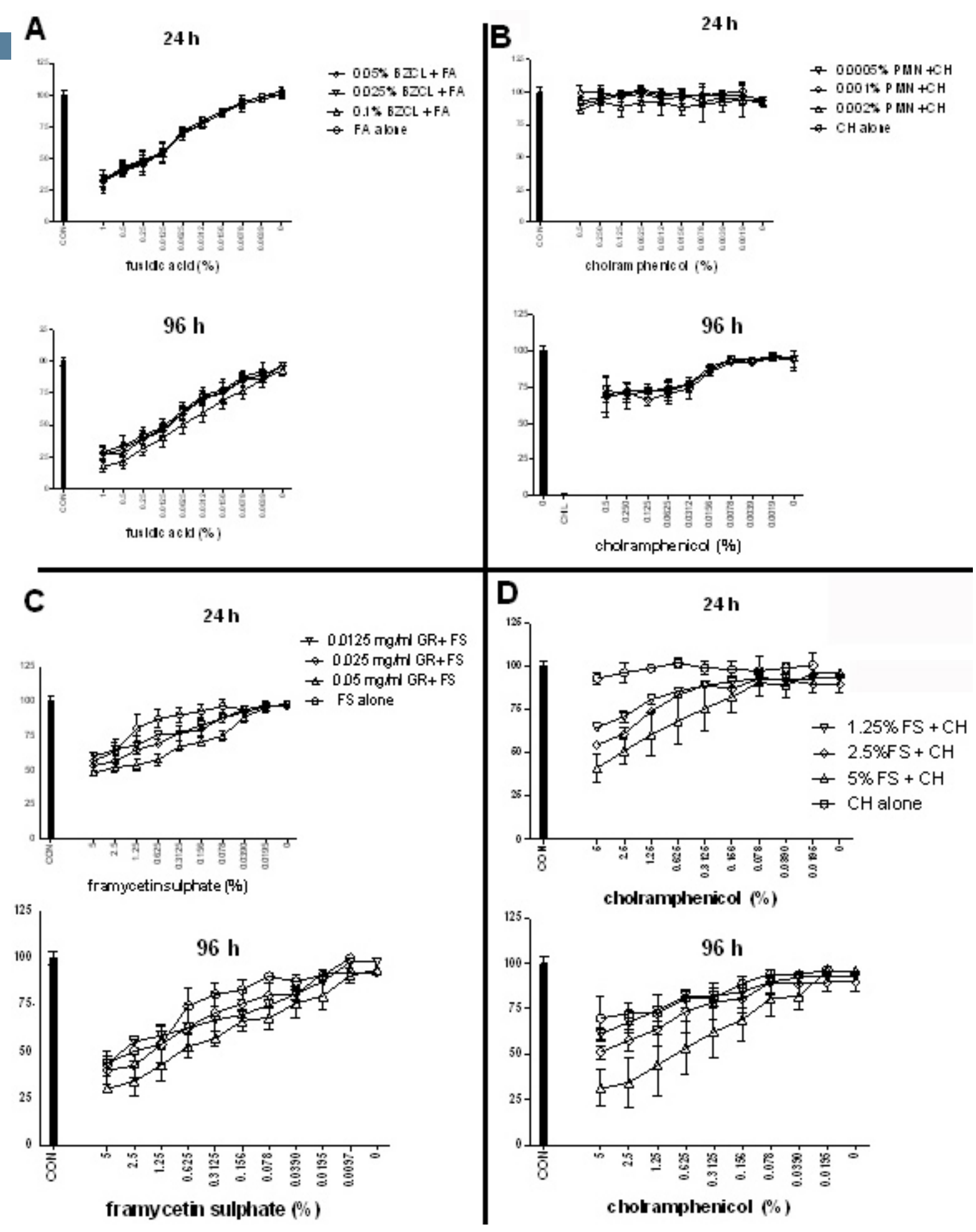

Figure 2: Percentage reduction of alamarBlue by A. polyphaga in the presence of combinations of excipients
Legend: A) fusidic acid and benzalkonium chloride, B) chloramphenicol and phenylmercuric nitrate, C) gramicidin and **framycetin sulfate, D) framycetin sulfate and chloramphenicol. Cultures carried out for a) 24 h and b) 96 h.**

error (SE). Statistical significance was calculated using the Mann-Whitney U test, with a p-value of <0.05 considered to be significant. Statistical analysis was performed using the GRAPH PAD PRISM 5 software.

Results

The addition of chloramphenicol to the A. polyphaga cultures resulted in a significant dose-dependent decrease in alamarBlue reduction by the cells, but only for the 96 hour test (Figure 1A). No inhibitory activity was found for the 24 hour culture. On the other hand, fusidic acid had a large inhibitory effect for both culture lengths, with the concentrations at which 50% inhibition was achieved (IC50 values) being 0.125% and 0.062% for the 24 hour and 96 hour experiments, respectively (Figure 1B).

Framycetin sulfate also displayed inhibitory activity, although this was only significant enough to calculate an IC50 when the culture was carried out for 96 hours (IC50: 5.0%; (Figure 1C). Gramicidin caused a level of inhibition for both culture durations, with an IC50 of 5.0% calculated for the 96 hour experiments (Figure 1D). Ciprofloxacin showed some activity at the highest concentrations, but this reached no more than a percentage reduction of alamarBlue of 85% (Figure 1E).

Neither benzalkonium chloride nor sodium carboxymethyl cellulose displayed any inhibitory activity against A. polyphaga at any concentration for either culture length (Figure 1 F and H). Phenylmercuric nitrate on the other hand, displayed a level of activity at the higher concentrations, with the effect being more pronounced for the 96 hour culture (Figure 1G). The effect was not significant enough for an IC50 to be calculated, however.

When different combinations of the eye drop excipients were tested for activity against A. polyphaga, the combination of fusidic acid and benzalkonium chloride (Figure 2A) appeared to provide much the same response to that of fusidic acid alone (Figure 1B). Again, high inhibitory activity was found for both culture lengths, with perhaps a slight increase in activity when the highest concentration of benzalkonium chloride was present for the 96 hour experiment. The combination of chloramphenicol and phenylmercuric nitrate produced no inhibition of the microorganism for the 24 hour culture (Figure 2B). For the 96 hour culture, however, some activity was evident at the higher chloramphenicol concentrations. The shape of the curves closely mirrored that of when phenylmercuric nitrate was tested alone (Figure 1G), but the concentration of this excipient had no effect on the level of inhibition.

Another combination of excipients that was tested was gramicidin with framycetin sulfate. When tested alone, both of these agents demonstrated inhibitory activity against A. polyphaga in both the 24 hour and 96 hour cultures (Figure 1C and D). Together, a small additive effect can be seen, with the level of inhibition being greatest when the highest concentrations of each excipient were used in combination (Figure 2C).

While chloramphenicol alone only showed inhibitory activity for the 96 hour culture, in combination with framycetin sulfate, activity was evident for both lengths of experiment (Figure 2D). For the shorter of the two cultures, the inhibitory activity was higher for the combination of excipients than that when framycetin was tested alone. The maximum level of alamarBlue reduction for the highest concentration of framycetin sulphate (5 mg/ml) was approximately 55%, while this decreased to around 40% when combined with 5% chloramphenicol. For the 96 hour culture, alamarBlue reduction reached a low of approximately 25% for the combination of the highest concentrations of the two excipients. This was much lower than the 70% and 45% found for chloramphenicol and framycetin sulfate alone, respectively (Figure 1A and C).

Discussion

There is an increasing need to develop novel agents against *A. polyphaga*, one of the protozoal species responsible for Acanthamoeba keratitis. The task of identifying such compounds is ongoing; however, to date, no study has evaluated the efficacy of agents already used in commercial eye drops. Having already been demonstrated to be safe for ophthalmologic use, such compounds could rapidly gain regulatory approval for the treatment of this potentially blinding condition.

Chloramphenicol displays broad bacteriostatic activity against both gram positive and gram negative bacteria by inhibiting protein synthesis via irreversible binding to the 50S subunit of the ribosome. It has long been used to treat bacterial conjunctivitis [14], and has recently demonstrated anti-yeast properties [15]. We found that alamarBlue reduction by *A. polyphaga* was inhibited by the compound, but only when the cells were cultured in its presence for 96 hours. This indicates that chloramphenicol works slowly against the organism, requiring a certain length of time in order to achieve an effect.

Fusidic acid is another bacteriostatic compound, displaying activity against gram positive bacteria. It works by inhibiting protein synthesis via prevention of turnover of elongation factor G from the ribosome [16], and is used in the treatment of bacterial conjunctivitis [17]. We found that the agent produced significant inhibitory activity against *A. polyphaga* in both the 24 hour and 96 hour cultures. This demonstrates that despite its narrow scope as an antibacterial, it is a promising candidate for the treatment of *Acanthamoeba* keratitis.

Framycetin sulfate is a broad spectrum aminoglycoside antibacterial that works by inhibiting protein synthesis via ribosomal binding. It is active against gram negative and some gram positive bacteria, but has not been demonstrated to have any antifungal activity. Whilst there are no reports on the effect of this agent on any protozoal species, another aminoglycoside antibacterial, paromomycin, has demonstrated activity against the *Leishmania* species of protozoa [18-20]. We found that framycetin sulfate inhibited alamarBlue reduction by

A. polyphaga, with a more significant effect evident for the longer 96 hour culture. The data therefore suggest that this compound is another potential therapy for *Acanthamoeba* keratitis, and warrants further study.

Gramicidin is an antibacterial that causes cell death by increasing the permeability of the cell membrane leading to leakage of small molecules such as monovalent ions and amino acids. To date, reports of the activity of gramicidin have been limited to gram positive bacteria, with no evidence of antifungal or antiprotozoal activity when used alone. Here, we found that the compound had a level of activity against *A. polyphaga*, with this being greater for the 96 hour culture in comparison with the 24 hour.

Ciprofloxacin is a fluoroquinolone broad spectrum antibacterial that is used to treat conjunctivitis, keratitis, and corneal ulcers [21]. It works by hindering cell division via inhibition of DNA topoisomerases [22]. The compound has also displayed activity against *Leishmania* or topoisomerases present in this organism [23, 24]. Here, we found that ciprofloxacin demonstrated a low level of activity against *A. polyphaga*, with similar activity profiles for the two different culture lengths. Such limited activity indicates that this compound would not be useful for the treatment of *Acanthamoeba* keratitis.

Benzalkonium chloride is a quaternary ammonium salt preservative used in many forms of eye drops and artificial tears. It works as an antibacterial by binding to the negatively charged cell membrane and increasing its permeability, resulting in leakage of monovalent ions and subsequently, cell death. No inhibitory activity against *A. polyphaga* was found in the present study, for either culture length. This is in contrast to the data published by Tu et al., who demonstrated significant in vitro activity of benzalkonium chloride against three species of Acanthamoeba, among them *A. polyphaga* [25). Activity was high after just an hour, even at concentrations much lower than those used in the present work. Zanetti et al. reported activity of the compound against *A. castellanii,* another organism responsible for *Acanthamoeba* keratitis, both in trophozoite form and cyst form [26]. These conflicting results suggest that further tests should be carried out before benzalkonium chloride is discounted as a treatment for the condition.

Phenylmercuric nitrate is another preservative used in eye drops. It displays both antibacterial and antifungal activity as a result of increasing the permeability of the cell membrane [27, 29]. We found that the compound was active against *A. polyphaga* at the higher concentrations, with a greater effect for the 96 hour culture. This indicates that phenylmercuric nitrate may be useful in the treatment of *Acanthamoeba* keratitis if the dosing can be sustained over a number of days.

The final eye drop excipient that we tested was the viscosity modifier, sodium carboxymethyl cellulose. As expected, this compound demonstrated no inhibitory activity against *A. polyphaga.*

Fusidic acid eye drops often contain benzalkonium chloride as a preservative; we therefore tested these two agents in combination in order to determine if there was an additive effect. The activity of the combination was generally the same as that of fusidic acid alone. The only exception to this was a slightly greater activity when the highest concentration of benzalkonium chloride was used in the 96 hour culture. Importantly, no detrimental effect was found, indicating that commercially available fusidic acid eye drops may be a potential treatment option for *Acanthamoeba* keratitis.

Another commonly found combination of agents in eye drops is chloramphenicol and phenylmercuric nitrate. For the 24 hour culture, this combination appeared to have no inhibitory activity against *A. polyphaga*, despite phenylmercuric nitrate alone showing some activity in the earlier experiments. This is likely due to the low concentrations of this compound that were added to the chloramphenicol for this particular test. The activity of the phenylmercuric nitrate was only found at the higher concentrations that were tested in the single compound experiment. It is also possible that the presence of chloramphenicol lowered the activity of the phenylmercuric nitrate. The data taken from the 96 hour culture also point to this possibility as the level of A. polyphaga inhibition was almost the same as that seen for the chloramphenicol alone, with no additional effect found on the addition of the phenylmercuric nitrate. This combination of excipients does not appear to be a potentially effective treatment for *Acanthamoeba* keratitis.

Gramicidin and framycetin sulfate are often used in combination for treating eye infections. We found that the inhibitory activity of this combination was greater than that found for either agent alone. For the 96 hour culture in particular, alamarBlue reduction decreased to approximately 25%, one of the lowest values found in the present study. The data therefore suggest that the commercially available eye drops that utilise this combination could be effectively used for the treatment of *Acanthamoeba*-based infections.

We also investigated the combination of framycetin sulfate and chloramphenicol. For the 24 hour culture, while chloramphenicol alone had not demonstrated any activity in the prior tests, on the addition of even a small amount of framycetin sulfate, there was an increase in A. polyphaga inhibition. This additive effect was even more evident for the 96 hour culture, with alamarBlue reduction decreasing to around 25% when the highest concentrations of the two compounds were used together. This combination of agents appears to be a promising treatment option for Acanthamoeba keratitis and warrants further evaluation.

Conclusions

We have demonstrated significant activity against *A. polyphaga* of a number of compounds that are currently used as active ingredients of preservatives in commercially available eye drops. These preliminary data indicate that

further investigation into some of these agents may lead to additional treatment options for *Acanthamoeba* keratitis. As these excipients have already been approved for ocular use, there is potential for new combinations of them to be rapidly introduced to the market.

Conflict Of Interest

The author of this publication receives research support from Public Authority for Agriculture Affairs and Fish Resources - Al-rabia, Kuwait City.. The terms of this arrangement have been reviewed and approved by the University of Kuwait in accordance with its policy on objectivity in research.

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Storage of Medicines and Temperature Control at Community Pharmacies in Rural District of Sindh, Pakistan: An Exploratory Cross-Sectional Study

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Abstract

Background: **Medicines are the essential tools for prevention, cure and control of diseases. If these medicines are ineffective then their aftermath can cause wastage of resources. Medicines lose their required effectiveness due to inadequate storage at required temperature.**

Objective: **The objective was to estimate the proportion of pharmacies with high temperature (>25°C) inside pharmacy outlets in two talukas (sub-districts) of district Thatta, Sindh**

Methodology: **An exploratory cross sectional study design was conducted from August 2013 to August 2014. All pharmacies of the two talukas were approached by doing a census. Descriptive analysis was done to calculate the frequencies and proportions.**

Results: **All pharmacies (n=62) had a temperature of >25°C inside the pharmacies. Medicines were exposed to sunlight in 39 (63%) of the pharmacies and 39 (63%) of pharmacies had refrigerators to keep insulin and vaccines. Median duration of electricity shut downs was 12 hours per day and 11% of the pharmacies had back up power supply.**

Conclusion: **More than a quarter of pharmacy owners were aware about maintaining the required temperature of < 25°C but none of them were maintaining required temperature. Considering the electricity shut down, it is important to make cost effective and long term strategies to maintain the efficacy of medicines. Proper legislation needs to be enforced with continuing training programs for pharmacy owners. Further research is required to explore different ways of maintaining required temperature to ensure the adequate efficacy of medicines.**

Key words: **Pharmacy, Temperature control, Storage, Medicines**

Introduction

Medicines including vaccines are considered as one of the important tools in combating diseases across the world, but these medicines may also cause adverse events with varying severity which usually depend upon patient as well as product related factors(1). This issue is even more critical in developing countries where most medications are not stored at appropriate conditions and can be easily purchased over the counter(1). Medicinal products require appropriate storage conditions in order to ensure the quality and efficacy of medicines (2). Medicines which are not stored on required temperature (< 25? C) can further increase unnecessary burden on economy of general population due to their ineffectiveness in curing of disease (2).

Literature shows that stability of pharmaceutical products is extremely essential to maintain their therapeutic efficacy(3). Temperature plays a key role to maintain the required efficacy of medicines (4-6). It has been suggested that more than 50% of medicines should be stored on required temperature, which is usually less than 25?C (6-8). Apart from high temperature (> 25°C), direct exposure to sunlight and improper management of medicines during shipment can also cause damage to pharmaceutical products like antivirals, multi-vitamins, acetaminophens, antibiotics, diuretics, hydrocortisone, eye drops, analgesics, anti-depressants and latex products such as male condoms (9, 10). Light can change the properties of different materials and products. This change in properties can be due to steadily increasing exposure of medicines to high temperature during storage or dispensing the medicines at the pharmacies through different mechanisms(11, 12).

Almost every country in the world has some mechanism of providing drugs to people residing in communities, either through proper prescription or without prescription of medications. These countries have retail and wholesale pharmacies working regularly to serve their community (1).

likewise other countries, there are an estimated 45 000 to 50 000 wholesale and retail drug outlets in Pakistan (1). Although there is a network of health services in Pakistan's public sector, and overabundance of private sector initiatives, 45% of the population still lacks access to health services (13). Thus, in order to meet health needs and to reduce out of pocket payment, people rely on alternative health care systems such as traditional medicine practitioners, chemists, faith healers, and homeopaths(1). Self-medication has been consistently increasing due to large gaps in the formal health sector of the country (1).

Furthermore, people usually rely on the nearby accessible medical stores or small community pharmacies to purchase medicines for different diseases (14, 15). In many countries, community pharmacies are places where people may obtain health advice and assistance to manage their disease states with required medications (16). Moreover, these pharmacies are often the first point of contact for patients seeking health care as they are usually more accessible and less socially distant than other providers including medical doctors (1). Community pharmacies have been considered as a key interface between the health care system and the general public (14, 15). It is common that many workers at community pharmacies are involved in providing health advice on most of the health problems prevailing in community (14, 15). Even most of the times, community pharmacy workers prescribe medicines, which are sometimes safe and effective when used correctly, however these can become dangerous in case of emergencies (17, 18). Community pharmacies are perceived as an easy and convenient source of advice, referral to nearby facility and source of required information by the patients and their families in developing world, including Pakistan (19, 20).

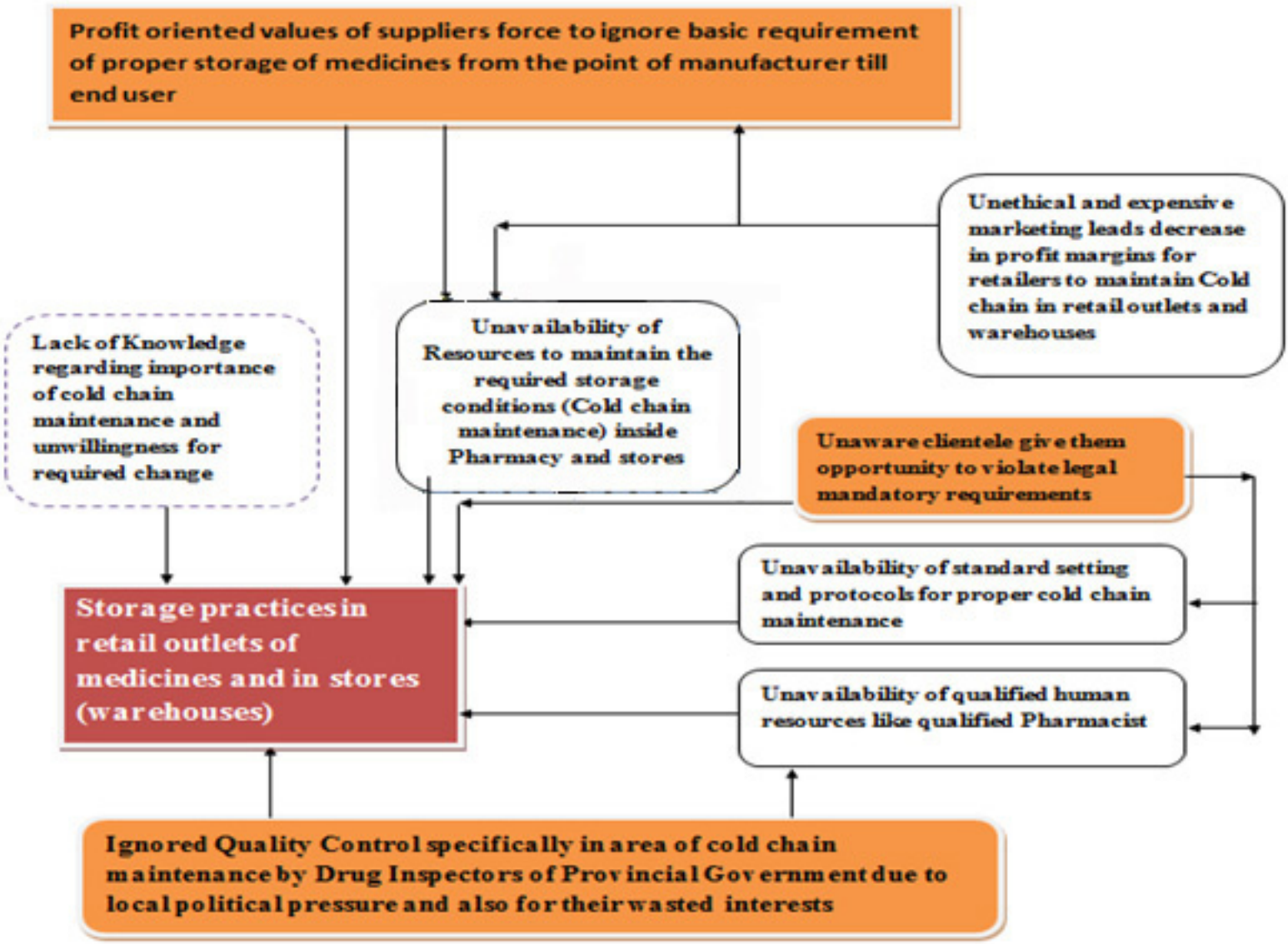
Community pharmacy owners can be considered as one of the important influential stakeholders in health care system who can affect the drug usage owing to their scale of operations and placement in the healthcare delivery system (21). Looking at importance of community pharmacies and their outreach services, many developing countries have used their potential to promote safe and effective treatments (17). This has been done by enhancing their dispensing practices and management of medicines inside pharmacy outlets and in stores, which are easily acceptable to community pharmacy workers and their owners (17).

In Pakistan unfortunately the area of community pharmacy has been ignored by policymakers to make collaboration of community pharmacy workers and owners with other stake holders in health sector of country for the betterment of population. Therefore it is essential to work in team by including community pharmacy workers for betterment of general population (22)

Thus it is essential to know the importance of community pharmacies in the context of temperature management during hot weather and particularly in rural areas of Pakistan (23).

Furthermore, in Pakistan there is an issue of increased electricity shut downs in rural areas even for more than 12 hours which prevents storing medicines at required temperature in community pharmacies of rural areas of Pakistan. Community pharmacies are one of the important sources of dispensing medicines to the Pakistani population. Knowledge about proper storage of medicines at required temperature in community pharmacies is not available. In country like Pakistan many other issues also arise in hot weather and it is a challenge to store the medicines appropriately on required temperature due to electricity shut downs and lack of backup power supply (23). Thus, it is important to know that whether the medicines are stored on required temperature or not, particularly in community pharmacies of rural areas.

Figure 1: Storage Practices in retail Pharmacies at District Level: A conceptual Framework



This study had assessed the practices regarding storage of medicines in rural area of Sindh, Pakistan. This study was aimed to estimate the proportion of pharmacies with high temperature (> 25 °C) inside the pharmacy outlets in Sindh Pakistan and to estimate the proportion of pharmacies selling vaccines, insulin and have refrigerators with and without backup power supply. This information will contribute to improve policies for managing and storing medicines appropriately especially in remote areas of Sindh.

Methods

Exploratory cross sectional study design was used to conduct this study form August 2013 to August 2014. This study was conducted in two areas of the Thatta district. These areas included two talukas (sub-districts), Sujawal and Jati. Thatta is a rural district in the southernmost part of Sindh province in Pakistan, bordering Karachi (24). The estimated population of district is around 1513194 (25) with literacy rate of 22.1% (26). There are five public sector hospitals in Thatta, as well as 8 rural health centers (RHCs) and 51 basic health units (BHUs).

Those community pharmacies which were selling at least allopathic medicines in the two talukas (sub-districts) of district Thatta (Sujawal and Jati) and whose pharmacy owners gave the written informed consent were included in this study. While those pharmacies, which were located in the hospital premises or where a medical doctor was practicing were excluded from this study.

We did a census for all pharmacies of two talukas (sub-districts) to get the list of eligible pharmacies. The census covered a total of 70 pharmacies and all 70 pharmacies and their pharmacy owners or drug sellers were approached but 8 pharmacies refused to give the informed consent thus could not be included in the study. Thus 62 pharmacies were included through consecutive non-probability sampling.

Pharmacy owners were interviewed through a structured and pretested questionnaire. A checklist was also used to assess the storage conditions in the community pharmacies during month of June and July 2014. Room thermometer was used to measure the temperature in all the selected pharmacies. Three digital thermometers and three mercury maximum/minimum thermometers were purchased and checked by comparing these thermometers with standard thermometer in biomedical department of Aga Khan University for 20 hours. The

Table 1: Knowledge of pharmacy owners about temperature and storage practices (n=62)

Characteristics	n (%)
Service provision in years by pharmacy owners [median (IQR)]	8 (6-10)
Knowledge about temperature of pharmacies (Inner half)	
Less than 25°C	18 (29.0%)
Equal to 25°C	2 (3.2%)
25°C to 30°C	41 (66.1%)
Equal to 30°C	1(1.6%)
Knowledge about temperature of pharmacies (Outer half)	
Less than 25°C	17 (27.4%)
Equal to 25°C	2 (3.2%)
25°C to 30°C	36 (58.1%)
Equal to 30°C	7 (11.3%)
Temperature monitoring	
Yes	2 (3.2%)
No	60 (96.8%)
Frequency of temperature monitoring	
Daily	1 (1.6%)
Weekly	1 (1.6%)
Calibrate the thermometer against certified thermometer	
Yes	0 (0%)
No	2 (3.2%)
Awareness about spoiling of medicines due to high temperature	
Yes	61(98.4%)
No	1 (1.6%)
knowledge about lethality of medicines due to sunlight exposure	
Antibiotics	39(62.9%)
NSAIDs	13(21.0%)
Anti-Allergic	10(16.1%)
Check outdated medicines	
Yes	60 (96.8%)
No	02 (3.2%)
Frequency of checking outdated medicines	
Weekly	11(17.7%)
Monthly	48(77.4%)
Quarterly	1 (1.6%)

biomedical department engineers agreed within standard deviation of 0.2°C. Two digital thermometers along with mercury thermometers were placed in every community pharmacy for half an hour. One thermometer was kept in outer half of the community pharmacy where first drug was placed and one in inner half of the community pharmacy along where last drug was placed. Over the same period of time maximum ambient air temperature of Karachi was also noted from website of Pakistan meteorological department, whose results displayed on different web pages. Three readings of the temperature were taken at three different timings of the day. The maximum temperature in the day was recorded at 1500 hours of each day. After taking the three readings, an average value of all readings was taken to overcome the intra rater bias.

Data was analyzed through SPSS version 19. Frequencies and proportions were generated for all the variables. Estimated prevalence of pharmacies with high temperature (>25 °C) was calculated. Descriptive statistics were computed for both continuous and categorical variables. This study was conducted only after ethical approval by Ethical Review Committee (ERC) of the Aga Khan University.

Results

From a total of 70 pharmacies, 62 pharmacy owners expressed their willingness to participate in the study, making the interview response rate as 95%. Median duration of service provision by pharmacy owners in the community was 8 years with interquartile range (IQR) of 6-10 years (Table 1).

With respect to knowledge about temperature of pharmacies, 18 (29%) of the pharmacy owners reported that temperature of inner half of pharmacy should be below 25 °C, 41 (66%) reported that temperature of inner half of pharmacy should be between 25 °C and 30 °C, 2 (3%) pharmacy owners reported that temperature should be equal to 25°C, and 1 (2%) reported that temperature should be equal to 30°C. With respect to the temperature of outer half of pharmacy, 17 (27%) of the pharmacy owners reported that temperature should be less than 25°C, 36 (58%) pharmacy owners said that it should be between 25°C and 30°C, 7 (11%) owners reported that temperature should be equal to 30°C, while 2 (3%) pharmacy owners reported that temperature should be equal to 25°C. Regarding temperature monitoring, 2 (3%) pharmacy owners reported that they used to monitor the temperature of main pharmacy with a room thermometer but none of them used to maintain the temperature of storage area up to required level. Out of these 2 pharmacies, 1 (2%) pharmacy owner reported to monitor the temperature on a daily basis while 1(2%) reported to monitor the temperature on a weekly basis. None of them used to calibrate the thermometer against the certified thermometer (Table 1).

With respect to knowledge about temperature of pharmacies, 18 (29%) of the pharmacy owners reported that temperature of inner half of pharmacy should be below 25 °C, 41 (66%) reported that temperature of inner half of pharmacy should be between 25 °C and 30 °C, 2 (3%) pharmacy owners reported that temperature should be equal to 25°C, and 1 (2%) reported that temperature should be equal to 30°C. With respect to the temperature of outer half of pharmacy, 17 (27%) of the pharmacy owners reported that temperature should be less than 25°C, 36 (58%) pharmacy owners said that it should be between 25°C and 30°C, 7 (11%) owners reported that temperature should be equal to 30°C, while 2 (3%) pharmacy owners reported that temperature should be equal to 25°C. Regarding temperature monitoring, 2 (3%) pharmacy owners reported that they used to monitor the temperature of main pharmacy with a room thermometer but none of them used to maintain the temperature of storage area up to required level. Out of these 2 pharmacies, 1 (2%) pharmacy owner reported to monitor the temperature on a daily basis while 1(2%) reported to monitor the temperature on a weekly basis. None of them used to calibrate the thermometer against the certified thermometer (Table 1).

Regarding the knowledge about toxic effects of medicines due to sunlight exposure, 39 (63%) of the pharmacy owners reported that antibiotics can become ineffective if exposed to sunlight, followed by 13 (21%) owners indicating NSAIDS become ineffective upon sunlight exposure (Table 1). Out of 62 pharmacies, 30 (48%) pharmacy owners reported selling of insulin; all of them used to keep the insulin in refrigerators. Sixteen (26%) pharmacy owners reported selling of vaccines and keeping them in refrigerators. Regarding electricity shut down, all owners reported that they face this problem in their respective talukas; its median duration being 12 hours per day (IQR: 12-14hrs) but only 7 (11%) pharmacies had a backup supply of power (Table 2).

Furthermore, 35 (56%) pharmacies were found to have items, grouped in amounts that are easy to count. With respect to expired medicines, all 62 pharmacy owners used to keep the items with short expiry dates in front, while those medicines which had long expiry date were placed at the back (“first expired first out” principle). 9 (15%) pharmacies were maintaining the record for the removal of items including date, time, witness and manner of removal (Table 3).

Approximately 1.6% of the pharmacies were found to have the poor quality items in the shelves and similar proportion had any overstocked items in the shelves (Table 3). Moreover, the floors, walls, sinks, benches, shelves, containers and dispensing bottles were found to be clean in about 59 (95%) pharmacies. Only 4 (6%) pharmacies had a clearly defined area for reception of products, and 3 (5%) pharmacy owners reported that drugs are not handled with bare hands (Table 3).

Table 2: Storage practices of the medicines required to be stored in cool place (n=6

Characteristics	n (%)
Store insulin in Refrigerator	
Yes	30 (48.4%)
No	32 (51.6%)
Store Vaccine in Refrigerator	
Yes	16 (25.8%)
No	46 (74.1%)
Electrical shut downs compromising storage	
Yes	62 (100%)
No	0 (0%)
Duration of load shedding in hours [median(IQR)]	12 (12-14)
Back up supply of power	
Yes	7 (11.3%)
No	55(88.7%)

All of the 62 pharmacies (100%) had a temperature of >25°C (Table 4). In 39 (63%) pharmacies, sunlight exposure to medicines was evident. Among the medicines exposed to sunlight, NSAIDs comprised the major proportion (51.2%), followed by antibiotics (38.4%) and multivitamins (20.5%).

Similarly, 39 (63%) pharmacies had refrigerators to keep the important medicines in cool environment but only 2 (3%) pharmacies had temperature-monitoring devices (Table 4). None of the pharmacies had a cold storage facility other than the refrigerator.

Out of 62 pharmacy owners, 16 (26%) had a separate storage area within the main pharmacy and in these areas the temperature was found to be more than 25 °C. Approximately 8 (13%) pharmacy owners reported that the store is large enough to keep all the supplies. Around 11 (18%) pharmacies had a storage room which was reserved only for pharmacy related functions, and 7 (11%) had adequate storage capacity in the warehouse for medicines and medical supplies. Moreover, in 6 (10%) pharmacies, the stores were in good condition. One (2%) pharmacy was found to have a store with cracks, holes and signs of water leakage. In 9 (15%) pharmacies, the stores had a ceiling in from which 5 (8%) were not in good condition. In 2 (3%) pharmacies, the stores were properly ventilated with appropriate air entry. Stores of 7 (11%) pharmacies were tidy with clean shelves, walls and did not have signs of any infestation. Furthermore, 15% of the stores did not have any expired items in their storage area (Table 5).

Medical supplies were stored neatly on the shelves and boxes in 82% of the pharmacies but shelves and boxes were raised off the floor in only 23% of the pharmacies. In 5 (8%) pharmacies, the supplies were categorized in various groups. None of the pharmacy owners had practice of storing the controlled substances in double locked storage space (Table 5).

Discussion

Exposure of medicines to high temperature in storage or in transport could reduce their efficacy of drugs including vaccines(11). The major factors which contribute to decreased efficacy of medicines after quality manufacturing are improper storage of medicines at undesirable temperature (22). The journey of medicines begins at the site of manufacturer and passes through warehouses, pharmacies and sometimes other environments before reaching the end user (20). Temperature conditions in earlier stages have received attention, but little work has been done in primary care settings and community pharmacy settings, both in developed and developing countries (20).

The findings of present study suggest that majority of pharmacy owners had knowledge about correct temperature for storing medicines at that temperature (<25?C) but almost 100% of the community pharmacies were having temperature (>25?C) degree centigrade, which is more than the required temperature for storing medicines safely. In both environments i.e. outer and inner half of pharmacies, medicines were exposed to temperature greater than (>25?C). These findings suggest that there is huge gap between knowledge of pharmacy owners and their practices because on one hand they highlighted that high temperature (>25?C) can affect quality of medicines and on other hand temperature control at required level (<25?C) is not ensured in community pharmacies of these rural areas. Moreover, these findings are supported by the fact that medicines were directly exposed to sunlight in 63% of the community pharmacies. These findings are consistent with other studies around the world (20).

Our study also examined storage practices in the community pharmacies. Although, 63% of the pharmacies had a refrigerator to keep the medicines in a cool environment but due to incessant electricity shut downs (estimated to

Table 3: Findings regarding storage of medicines in main pharmacy

Characteristics	n=62
Arrangement of medicines on the shelves	
In Alphabetical order	01(1.6%)
Not in Alphabetical order	61(98.4%)
Items are grouped in amounts that are easy to count	35(56.5%)
Yes	27(43.5%)
No	
Medicines with short expiry dates in front of medicines with long expiry date	
Yes	62(100%)
No	0(0%)
Medicines were stored on the first expired first out principle	
Yes	53 (85.5%)
No	09(14.5%)
Poor quality items in the shelves	
Yes	1(1.6%)
No	61(98.4%)
Overstocked items in the shelves	
Yes	1(1.6%)
No	61(98.4%)
Pharmacy area was free from moisture	
Yes	59 (95.2%)
No	03 (4.8%)
Clearly defined and separated area for reception of products	
Yes	04 (6.5%)
No	58(93.5%)
Clearly defined and separated storage area for inflammable products	
Yes	1(1.6%)
No	61(98.4%)
Drugs handling	
With bare hands	59(95.1%)
Without bare hands	3 (4.8%)
Record maintenance	
Yes	09 (14.5%)
No	53(85.4%)

Table 4: Findings regarding the temperature of community pharmacies (Objective assessment) (n=62)

Characteristics	n(%)
Temperature of inner half of Pharmacy	
30°C to 35°C	62 (100%)
< 30°C	0 (0%)
Temperature of outer half of Pharmacy	
30°C to 35°C	62 (100%)
< 30°C	0 (0%)
Stores in Pharmacy	
Yes	16 (25.8%)
No	46 (74.2%)
Temperature inside stores of pharmacy	
30°C to 35°C	16 (100%)
< 30°C	0 (0%)
Exposure of medicines to sun light	
Yes	39 (62.9%)
No	23 (37.1%)
Use of refrigerator	
Yes	39 (62.9%)
No	23 (37.1%)
Cold storage facility other than refrigerator	
Yes	0 (0%)
No	62 (100%)

be approximately 12 hours per day), and limited backup power supply, may render these medicines ineffective.

Our study reveals that 3% of the pharmacies were having a temperature monitoring facility or device. With a dearth of temperature monitoring devices, excessive electric cut-offs and limited alternative power supply for refrigeration, and may potentiate doubt on the efficacy of medicines. These findings were consistent with that reported from Banglore, India (27). These findings of having refrigerator and temperature monitoring facility were slightly different from the study findings conducted by Zahid A Bhutt et al in Urban Rawalpindi in 2005, where majority (76%) of the pharmacy owners had refrigerator and 10% of these pharmacies had temperature monitoring devices as well(28). The difference in findings of having refrigerator in current study might be due to setting of the study in the rural district of Sindh where worth of purchasing refrigerator might not be considered as compared to urban areas. Moreover, due to frequent electricity cut- offs, pharmacy owners might not prefer to invest in purchasing the refrigerator or they might not have the knowledge of keeping the required medicines in the refrigerator.

Around 25% of the pharmacies were selling vaccines and all of these pharmacies were storing the vaccines in refrigerator, despite limitations of maintaining the desired temperature. These findings were different from the studies conducted in Karachi and Rawalpindi, where more than half of the pharmacy owners were selling vaccines irrespective of appropriate storage practices(28, 29). This difference might be due to difference in demand and supply of rural and urban areas. In urban areas, community people might be aware about and might give high importance of preventive aspect of the health therefore there would be more demand for vaccines, which might be satisfied by equal supply by community pharmacies in urban areas.

Furthermore, our study also found that commonly used medicines like NSAIDs, multivitamins and antibiotics were exposed to direct sunlight and studies have also shown that such medicines show significant reductions in activity when stored at temperature more than 25oC (20). Furthermore, studies have also shown that dissolution rate of diclofenac sodium (NSAIDs) tablets significantly reduces in as little as three months following exposure to high ambient temperature(20). Although the community pharmacy owners had knowledge about the adverse effects of high temperature on medicines but they could not modify the temperature of community pharmacies alone due to multiple barriers like lack of air conditioning system, load shedding problems and lack of backup power supply in community pharmacies. Moreover, there might be other barriers or factors which might have stopped community pharmacy owners or drug sellers to maintain the required temperature in their respective pharmacies and there is strong need to explore such barriers in future studies.

Table 5: Findings regarding storage of medicines in separate storage area of main Pharmacy

Characteristics	n=62
Separate storage area in main pharmacy	
Yes	16 (25.8%)
No	46 (74.1%)
Store is large to keep all the supplies	
Yes	08 (12.9%)
No	54 (87.0%)
Storage room reserved only for pharmacy related functions	
Yes	11 (17.7%)
No	51(82.2%)
Adequate storage capacity in the warehouse for medicines	
Yes	07 (11.3%)
No	55(88.7%)
Stores were in good condition	
Yes	6 (9.7%)
No	56 (90.3%)
Proper ventilation in stores	
Yes	2 (3.2%)
No	60 (96.2%)
Signs of pest infestations	
Yes	55 (88.7%)
No	07 (11.3%)
Stores were tidy	
Yes	55 (88.7%)
No	07 (11.3%)
Stores with clean shelves and walls	
Yes	55 (88.7%)
No	07 (11.3%)
Medical supplies were stored neatly on the shelves and boxes	
Yes	51 (82.3%)
No	11 (17.4%)
Shelves and boxes were raised off floor	
Yes	14 (22.6%)
No	48 (77.4%)
Medicines shaped in groups: external, internals and injectables	
Yes	5 (8.1%)
No	57 (91.9%)

Since pharmacies are often the first point of interaction for patients looking for health care as they are usually more reachable and less socially distant than other health care providers, including general physicians and consultants. Therefore, if drugs or vaccines are compromised by quality issues such as improper storage of medicines at undesirable temperature then community people may end up with products or instructions that are useless and even dangerous for the community as a whole(28). In fact, it has been said that if a physician's medical error could terminate a life, then a singular error from the drug manufacturer or dealer can most certainly lead to the loss of many lives(30).

Strengths and Limitations of the Study

There is a scarcity of literature regarding the storage practices of medicines in community pharmacies, particularly in rural areas of Pakistan. Therefore, to the best of our knowledge this was the first study of its kind that has assessed the temperature and storage practices of medicines in community pharmacies in rural district of Sindh, Pakistan. Temperature was measured objectively with the standard procedure to avoid the measurement bias. In addition to this, study findings can be generalized to rural areas of developing countries.

The scope of this study was limited to pharmacy owners and drug inspectors but perceptions of owners of pharmaceutical companies, other government authorities, community stakeholders and policy makers could not be assessed. The collection of data was limited to only two talukas. Interviewer bias might be there due to nature of questions being asked from pharmacy owners.

Conclusions

The study found more than a quarter of pharmacy owners had knowledge about maintaining the required temperature of < 25°C but none of the pharmacies in the catchment area were maintaining required temperature of < 25°C. We also concluded that storage practices in community pharmacies / medicine stores were found to be poor and very few pharmacy owners were monitoring temperature in their pharmacies. Although there were some observed insignificant cases of satisfactory storage practices amongst community pharmacies/ medicine stores, nevertheless the evidently generally poor storage practices weighed higher because of their potential untoward chain effects on drug consumers.

Moreover, looking at the median duration of load shedding and limited backup power supply, it is very important to make cost effective and long lasting strategies to maintain the safety, efficacy of medicines and to improve quality of medicines especially in remote areas. Strict monitoring and regulation of these pharmacies are required. Such storage practices should also be evaluated at homes to see that how people store these medicines in their households. Furthermore, there is a need to enforce existing legislation with ongoing training programs directed towards pharmacy owners and drug sellers and to involve the pharmaceutical industry, which plays an important role in influencing pharmacy practices of pharmacy owners. In future, more research is required to explore the different ways of maintaining the required storage conditions of medicines to ensure the adequate efficacy, safety and effectiveness of medicines.

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Plagiarism and Self plagiarism from the perspective of academic authors

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Abstract

This paper looks at the background and history of plagiarism and self plagiarism, reviews aspects of academic self plagiarism from the academic, the institution and publisher's point of view and provides a handy check-list of the current definitions and requirements.

Key words: plagiarism, self plagiarism

History and Background

The concept of plagiarism goes back many centuries. The word plagiarism derives from Latin roots: plagiarius, an abductor, and plagiare, to steal. The first recorded case of plagiarism was by the roman poet Martial who lived from 40 AD to somewhere between 102 and 104 AD. Prior to that the concept was seen in a positive manner as a way of passing down and disseminating great works of literature or art. This likely carried on the previous tradition of humans passing down histories and ideas by word of mouth. (1)

Written material like religious texts were once freely copied and incorporated into later works, and good writing usually meant slavishly imitating a small number of respected authors. However, poets, and playwrights tended to protect their original works. (1,2,3,4)

During the Renaissance, original scholarship became more respected and individual accomplishment was recognized in many more fields than it had been previously (for example, this is when painters began signing their works). By the mid 1600s, accusations of plagiarism and stealing ideas were common in every creative field including the sciences. (1,2,3,4)

The modern concept of plagiarism as immoral, and originality as an ideal, emerged in Europe only in the 18th century, particularly with the Romantic Movement which then extended the idea to art and the visual image. (1,2,3,4)

The first English copyright law was passed in 1709. It had as much to do with protecting the rights of publishers against book piracy as it did with protecting the author's rights against unscrupulous printers, but authors' rights developed very quickly.(5)

However the precise definitions of plagiarism evolved during the 20th century. The word "plagiarism," in the sense we use it today, first appeared in English in the various battles among Shakespeare and his peers. The Oxford English Dictionary credits Ben Jonson with being the first to use it in print. The word they used was "plagiary," which is a Latin term for a type of kidnapper or illegitimate slaver. (5)

While the concept of plagiarism has generally been positively accepted, one of the most famous cases of the adverse effects of plagiarism on highly reputable and well intentioned authors involved Charles Darwin in his publishing of "The Origin of the Species" in 1859.

Alfred Russel Wallace, a contemporary of Darwin was also independently working on the same issues: disease and famine, what kept human and other populations in check, recent discoveries, particularly newly observed fossil evidence showing the tremendous age of earth, and how this affected species over great periods of time. (6)

Wallace, in what can be seen as a huge strategic mistake on his part, wrote up and sent his ideas to Charles Darwin who was also a naturalist of great repute. Darwin had also been working on the same issues for decades, but vacillating about publishing due to some of the more controversial aspects of his work, namely the evolution of humans themselves, and decided to quickly publish and get his work out before Wallace (6). The rest is history. This is a prime example of the maxim 'publish or perish' and while both authors/researchers had original work and had high integrity, the process itself made one a winner and one a loser.

Current definitions of Plagiarism and Self Plagiarism

While there is some conjecture and controversy currently as to the precise definition and interpretation of plagiarism and self plagiarism, the established protocols, used and recommended by most current academic journals can be found in the Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals www.icmje.org and the COPE Code of Conduct http://publicationethics.org/files/u2/New_Code.pdf. (7, 8) These are usually displayed on the websites of each academic journal as Author Information or Instructions to Authors.

The definition of plagiarism is more straightforward, deliberate theft of another's intellectual property, however the definition of self plagiarism is a totally different issue and one that encompasses many issues both within the influence of the author and those which the author has no direct say over. It can also be subjective and not without bias or legal and economic sequelae.

On the one hand there are some who would argue that using large sections of text from one or more previously published papers in a paper presented as 'original', is almost fraudulent (9).

The New England Journal of Medicine insists that Authors should submit to the Editor copies of any published papers or other manuscripts in preparation or submitted elsewhere that are related to the manuscript to be considered by the journal. However, it is clearly unacceptable to submit the same paper to two different journals with the intention of the paper being perceived as two separate, original pieces. (9)

Publishers and Self Plagiarism

While assuming that most publishers are inherently reputable and have their own valid concerns they also have to deal in the legal (and subsequent economic sequelae) of copyright. This is further compounded in academic publishing by the re-use of journal articles in the major databases under exclusive or non-exclusive contracts.

What is deemed unacceptable duplication may contravene copyright law or violate copyright licenses. It is one of the reasons that when issues of plagiarism arise that the publishers firstly get together with the author and the involved academic institution if there is one, and try to sort out the problem between themselves before it becomes a point of law. A resolution is usually argued successfully and usually results in one or more of the publishing houses withdrawing that publication. The academic institutions involved then take their own measures internally.

Copyright of an academic's work is normally transferred to the publisher as a requirement of publication in scientific, medical and academic journals. Therefore plagiarism and self plagiarism is a copyright violation and the publisher concerned is the one legally liable, unless they have grounds to sue the other party or parties.

It is the obvious reason why plagiarism and self plagiarism can tend to be a punitive rather than a conciliatory approach on behalf of publishers. A 'winner' is usually decided upon and that winner retains copyright of the given work.

Self-plagiarism takes on yet another dimension as an issue of integrity, additional to the legal and copyright concerns of publishing houses. The reputation of the academic publishing house (or academic institution) can be diminished if they are seen to publish repetitive and non original material even though papers may have been deemed to be original at the time of acceptance. Electronic software can be used by such publishing houses and we will discuss this further on, but this does not get around issues of submission of the manuscript concurrently to two different publishing houses, or unintentional plagiarism or self plagiarism and indeed electronic software can encourage deliberate concealment of aspects of similarity.

The reputable academic author is best advised to discuss any concerns on these matters with their academic institution and the preferred publisher prior to, or at time of submission. They should also try to do as much research and cover as much ground as possible to ensure their work

is original, but allowing for the fact that some academics will have access to greater information resources than others.

The other obvious aspect is full attribution of all other sources of their material be it their own earlier publications or references from other works, as far as is humanly possible. Others, academicians and publishers, can then judge for themselves, prior to publication if they find the use of those sources admissable or not.

These same parties, academic institutions and publishers, should, have a wider and greater knowledge of the existence of other works on the same topic than the author, and will have their own resources to consult.

An academic publishers' editorial on this topic states "Self plagiarism comes down to the central issue of deception, were the authors trying to deceive the editors, the referees, and the readers into presenting recycled data, text and figures as entirely new material ?"(10)

Detecting plagiarism

Electronic software can provide users with a 'copying' and 'similarity' report through online searches and the most widely used is "Turnitin" (11). While these are good first line tools for publishers, institutions and academics they can actually encourage fraud and plagiarism, including self plagiarism. A quick check by running your material through such software, readily highlights what needs to be changed or paraphrased. This does not alter the content, or the source of the content, rather it assists in veiling it. An articulate person, such as an academic, can be quite skilled at re-presenting written work.

While a good first line tool, the issue goes way beyond running work through electronic software. Electronic software while giving a lot of detail does not show 'intention to deceive' and much implied deception, especially in the non English speaking world, can come down to lack of language skills and lack of availability of proper and relevant information and assumed inherent knowledge (17).

Attribution, referencing, showing sources and particularly discussion with all parties concerned would be the ideal approach and this currently happens to a fair degree but it cannot show intention to deceive. Currently the best and fairest way to do that would be psychological assessment and indeed a court of law. For practical reasons this does not occur, leaving the process somewhat subjective and open to bias or influence.

Dealing with plagiarism and misconduct

While details of dealings and consequences can be found clearly and in full in journals and on their websites the current accepted processes are listed as follows:

Pursuing misconduct by Editors
Editors have a duty to act if they suspect misconduct. This duty extends to both published and unpublished papers. Editors should not simply reject papers that raise concerns about possible misconduct. They are ethically obliged to pursue alleged cases.

Editors should first seek a response from those accused. If they are not satisfied with the response, they should ask the relevant employers or some appropriate body (perhaps a regulatory body) to investigate.

Editors should follow the COPE flowcharts where applicable (7,8).

Editors should make all reasonable efforts to ensure that a proper investigation is conducted; if this does not happen, Editors should make all reasonable attempts to persist in obtaining a resolution to the problem. This is an onerous but important duty.

Ensuring the integrity of the academic record: Whenever it is recognised that a significant inaccuracy, misleading statement or distorted report has been published, it must be corrected promptly and with due prominence. If, after an appropriate investigation, an item proves to be fraudulent, it should be retracted. The retraction should be clearly identifiable to readers and indexing systems.

Relations with journal owners and publishers.
The relationship of Editors to publishers and owners is often complex but should in each case be based firmly on the principle of Editorial independence. Notwithstanding the economic and political realities of their journals, Editors should make decisions on which articles to publish based on quality and suitability for readers rather than for immediate financial or political gain. (7,8)

Discussion

If the intention to deceive is the defining quality, especially when it comes to something nebulous like writing up of "ideas and knowledge" we are right to ask, who is qualified to judge? It would seem the job of a psychologist or an expert legal team in defining the intention to deceive if maximum fairness is to be achieved. This can happen. If plagiarism or self plagiarism is unintended authors can still feasibly have their work rejected on other grounds, legal and copyright. People's lives and livelihood are at stake in these cases. If there is no 'direct evidence' of intention to deceive (one way or the other) rulings can only be subjective. The rule of law adopted by most countries is the assumption of innocence until guilt is proved. The International Copyright Act under which most journals are published, while inherently sensible is somewhat more mechanical and driven by process, rather than relying on absolute truth.

"Ireland's (2009) editorial guidance to authors, whose work has been initially rejected by reviewers, may be useful in this context. Ireland states that for a paper to be considered

a 'new submission', it must meet all three of the following criteria: "(1) address modified or new research questions, (2) use new theoretical arguments, and (3) use additional or new data to test the proposed relationships (Ireland 2009, p. 10)." (9)

In seeking a definition of self-plagiarism in an Australian pilot study, lack of clear guidelines led the publishers to rely on the concept of 'fair use' according to the Australian Copyright Act which considers 10% textual re-use as acceptable. The British Medical Journal also uses a baseline of 10%, by requiring authors to send previous publications that overlap by more than 10%. (9,12)

The above quotes are a question in point. They do not identify 'intention to deceive' and do not clearly identify the purpose or type of article, or the purpose or the way in which the material has been re-used, rather it is an arbitrary percentage, a convenient process.

In essence if we are going to be completely fair to all concerned then each case of student, academic or commercial plagiarism, needs to be worked out on its own merits in a court of law.

Marking a student or academic 'down' for perceived and subjective intentional plagiarism can be just as much a crime against them and their future prospects in life and their academic reputations so should never be less than fully studied.

Unfortunately most of this is argument and conjecture and does not provide an answer - rather it shows that without an exact and legal process that is fully adhered to and consistent across all academic institutions and academic publications, there is no answer and the system may be flawed and subjective as a result.

Also the heavy requirements on academics to publish whether there is research of any importance or repute happening at their institution or if there is anything of significant merit to report or not, may be causing the problem. Maybe there should be less emphasis on number of publications and more on the merit of publishing particular research or study.

While deliberate fraud is unacceptable one major and seemingly obvious issue argued here is the assumed deception by authors in self plagiarism cases, when we should assume the opposite, that most academic authors have high integrity. It could be argued that some aspects of what is deemed self plagiarism may in fact be restrictions of academics' rights (restriction of fair trade) by institutions and publishers along with normal human issues, like impaired memory over time. Additionally an author may find very valid reasons to build on and further develop a work already done. Such is the way in the pursuit of knowledge.

Regarding who is qualified to judge the intention to deceive, currently it seems to be the one seen to have the most to gain or lose from that judgement, e.g. the university or journal to which the paper was submitted.

Too often authors are left in legal and copyright limbo and there has been little to no discussion on the rights of the academic author. Most arguments relate to legal and copyright issues of publishers.

There are very few 'new ideas' in this world. Indeed in medicine, Updates are an essential part of medical practice, as new medical techniques and therapeutics, are devised and Continuing Medical Education and proper patient care depend on this constant re-assessment. The 'shelf life' of medical education and publications therefore is deemed to be 2-5 years from a medical publisher's perspective. If the time factor in this case was also incorporated with the 10% re-use limit there should be a different set of rules for plagiarising in the medical publishing field. An author may be remiss not to re-visit their own and other's previous published work.

Rather than labelling re-use of what is essentially ongoing research and development, i.e. an author's intellectual property and ideas, as academic fraud, we may need more common sense and justice applied.

There seems to be some consensus that 'individual' or 'manual' assessment of an academic's publication is the preferred route where a multiplicity of factors can be viewed, but this does not help the academic author prior to the writing of the paper and the 'personal', manual' approach is still subjective and open to bias, be it academic or personal bias or publisher bias.

Authors therefore need to be seen less as potential criminals, rather, the positive aspects of clearly presenting their (new) work and making it easier for them to adapt to the many situations academic authors face, should be the focus from all concerned in the process. Evaluation of what is self plagiarism still carries a certain amount of luck over fairness. If an author is writing in a particular field and necessarily using commonly accepted jargon and terms, a simple similarity assessment is hardly solid evidence.

We may be stifling aspects of growth and development in certain disciplines accordingly, just so an institution can 'tabulate' an academic's progress within the academic promotional system. Additionally novel work may be deliberately stymied due to lack of promotional advancement opportunities within an institution e.g. there may be more than enough Professors in that discipline already at that institution. There may also be more or less opportunities, professional or financial resources, within any given institution for academics to develop their ideas and protocols to meet arbitrary judgements.

Are the needs of universities and publishers for example, the needs of academics and their students - not necessarily.

Is no idea worthy of going into print somewhere unless it is say 90% different to another idea. Ongoing thought is

natural and we are all influenced by outside forces and other's knowledge thoughts and opinions. And this makes the topic of self plagiarism a much bigger topic. Thought can be developed and refined over time.

Life is a natural and continual process of learning and while we need to attribute academic work and development fairly we also need to recognise and accommodate the realities. Innovation mainly comes from reviewing, applying, developing and refining knowledge and processes.

It is well known that the human mind can be quite deceptive, particularly so, to those who own that mind. Neuroscientists have shown that each time we remember something, we are reconstructing the event, reassembling it from traces throughout the brain. Psychologists have pointed out that we also suppress memories that are painful or damaging to self-esteem. We could say that, as a result, memory is unreliable. We could also say it is adaptive, reshaping itself to accommodate the new situations we find ourselves facing. (13, 14, 15)

Also what is the correct copyright and reference attribution to education and ideas. We remember many facts from our university days. Do we have to evaluate our lifetime's education and attribute and reference it. It can verge on gross silliness taken to extremes. The facts we learnt at school and university, in general press, news items and documentaries tend to be lumped together in the memory. This makes it very hard to judge intention to deceive if the mind itself cannot place facts and may alter them over time.

Currently academic institutions and publishers control the processes and hand out the judgments. We all need to rely on the integrity of academic publications and not waste our or authors' time in reinventing the wheel, however these matters should not be judged flippantly and proper process as well as reasoning needs to be applied.

Recommendations

The best way for academics to avoid plagiarism and self plagiarism particularly, is to publish original research or original developments or substantial updates on existing research. This does not get around the problem of other authors concurrently working on the same topics however if you have done your own research and have the data to show, your intention not to deceive should be plain. It is a common practice in academic publishing to also look at other studies and compare your results with theirs - whether they agree or disagree. Most of the problems may be circumvented by making your full intentions clear in the abstract and to outline how you particularly got to your conclusions, the date that these processes occurred and whether it was previous work, new work and developments on existing work.

If you anticipate some resistance from your academic institution there should be a method or protocol within that institution to properly evaluate the project prior to writing up the results and subsequent paper.

We provide our own checklist as follows:

Recommendations Checklist

1. Firstly check the author information on the website of the journal you are submitting to. They should have a complete list of author requirements.
2. Also if English is your second language consult if necessary a qualified English speaker and writer both to fully explain written requirements and to check the manuscript itself. There may be well known cultural references in other languages that are not familiar to you.
3. Keep and date all your original work, research, surveys, data collected and so on.
4. Make sure your abstract outlines the process of how you gathered the data for your article. If it is, for example, an argumentative essay and you have not overtly referenced any other published work or your previous work, still check that you or others have not previously covered this ground. If you have, attribute it and verify for yourself that your new work is not designed for the same purpose or to come to the same conclusions.
5. Make sure you have attributed/referenced any work, directly taken from your previous articles, or another publication or author.
6. If you are unsure that your work is not self plagiarism check with your academic institutions and peers about any concerns you may have.
7. If still unsure, seek the opinion of the journal you intend and wish to publish with and obtain their opinion and outline any concerns you may have, presenting your data and processes at the same time.
8. Don't send your paper to multiple publishers at the same time. If concerned about any possible delays in receiving an answer from your chosen publisher do prior research on how long the review and evaluation process takes at your chosen publishing house.
9. Finally, if you are acting with full integrity you should not have many concerns. If it turns out that you have inadvertently plagiarised or self plagiarised because of the many issues we have discussed in this paper, be full and frank with those publishers and your academic institution, and also remind them of your own legal and ethical approach and your own rights. You should not be assumed guilty unless proven to be so.

Recommended reading

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Introduction to Skin Surgery in the Office - some practical tips

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Introduction

This series in MEJFM reflects my experiences as a surgeon surgeon and thep roblems I have encountered over many years performing, photographing, videoing and teaching surgery in the office. Here I am attempting to impart some of the knowledge I have learned in a practical rather than theoretical sense.This is my approach to the issues I have confronted in my role as an educator and in designing and conducting surgical office skills workshops in skills laboratories throughout Australia and internationally. These have been on behalf of the Royal Australian College of Surgeons, Monash University, Queensland University and many private organizations including those associated with rural medicine and international medical graduates(IMG). In Australia this comprises up to 25% of the primary care workforce.

The titles EXPLORING ESSENTIAL SURGERY, EXPLORING ESSENTIAL RADIOLOGY are co authored with Dr Gerry Ahern and are prominently positioned on the McGraw Hill Access surgery and medical subscription websites.

In the workshops I have found that attendees like best hearing about his mistakes. But from these errors of judgment I have adopted techniques to overcome the problem.

I am going to take you on this journey in ensuing articles using the visual medium to enhance the process. The series is titled: Brygels SURGISKILLS.

Local anaesthesia, knot tying, suturing materials, suturing methods and tissue handling are the introductory topics. Then there are a whole range of topics including all the skin cancers, sebaceous cysts and lipomata, Ingrowing toenails.

Pre operative assessment and post operative care are all considered.

When surgery is performed on any skin lesion or subcutaneous lump, the aim is:

a. To completely excise the lesion and therefore provide optimal treatment and cure.
b. To preserve normal local function.
c. To obtain a good cosmetic result.

These principles should be adhered to in all other aspects of skin surgery, such for example, the drainage of an abscess, the removal of foreign bodies, the management of lacerations and trauma.
The primary care practitioner can provide appropriate treatment and management of all these conditions.

Excision of skin lesions, management of lacerations and trauma, plus treatment of infection and abscess are among the most common type of problem encountered in general medical practice.

Skin has a basic structure but there are specific characteristics in each body area which need to be taken account of in surgery.

The properties of skin which may alter from one site to the other and from one person to another.

The properties of skin to be considered are as follows:
• Thickness, stress lines, creases, blood supply, elasticity, mobility.
• The relationship to underling vital structures such as nerves ,vessels tendons and joints is of the utmost importance

All of these influence the method used in surgical operations. Each area of the body has its own particular characteristics.

All of these influence the method used in surgical operations. Each area of the body has its own particular characteristics.

For example:
• The blood supply is poor on the shin where the skin is thin particularly in the elderly. This influences how to treat or repair pretibial lacerations where suturing may be under tension and thus followed by tissue necrosis.

• The skin is thick on the back. As well the distracting forces are marked. Thus heavier sutures are required - may be a 2/0 suture as opposed to a 4/0 suture. The sutures may even be left in for 2 weeks. Maybe remove half earlier. For 3 months the scar will look perfect and fine ,but it often then stretches despite expert post operative care.

On the other hand skin is lax and mobile on the dorsum of the hand and much easier to incise, suture and close a defect without tension.

The skin over the sternum has a propensity for keloid formation. An incision on the chest wall is notorious for developing a keloid. Race is also a significant factor here. Thus experience alerts you immediately to the problem you may encounter in any particular area

Factors that must be considered when making decisions on the method of treatment. There are many factors that influence the decision to operate and the choice of method of treatment. Decisions are often influenced by the patient's age, ethnicity, community values, emotional status, patient expectations and associated medical problems. Cost and convenience may also be factors.

In addition there is often more than one way to treat a lesion. This needs to be explained to the patient from a risk management point of view. This is part of the informed consent process. An example here would be for basal cell carcinoma where there may be a choice between surgery, radiotherapy, photo dynamic therapy and curetting depending on the type and number of lesions, and even the training of the doctor.

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Trauma

The program has been designed to demonstrate a diagnostic approach using the methodology of taking a history and eliciting physical signs.

This has been included in this program because the fundamentals of management are important to all practitioners.

Cases of tetanus still occur. Many cases occur from simple gardening injuries. It is becoming apparent that even in our modern society, immunisation is still a necessity.

The correct treatment of all wounds is essential. The principles and practical aspects of management should be learnt by all those in the medical field.

As with any lesion, abscess or foreign body, a history of the onset, mechanism of injury and total assessment of the patient will avoid possible mistaken diagnosis and adverse sequelae.

Trauma does not only involve the skin. It can lead to damage to deeper structures, resulting in devitalisation of the tissues and possible injuries to nerve, arteries, veins, muscles, tendons, bone or other structures.

Infection still remains one of the major hazards of wounds and surgical operations. With any laceration, the priority is removal of foreign matter and devitalised tissue. This mechanical treatment which is important in prevention of infection, is termed debridement.

Judgement, based on training and experience, is essential in appropriate management for all wounds. A decision may be required as to whether the wound can or should be closed and if not, how the wound should be covered.

Anaerobic conditions in ischaemic or contaminated tissues can lead to the development of tetanus, or gas gangrene, particularly in the medically compromised patient.

The prevalence of hepatitis B, hepatitis C and AIDs has drawn attention to the risk to which the health worker is exposed. Increasing protection is being sought. Infection control routines have been set up in most clinical practices. Gloves, masks and goggles have assumed a new role in clinical practice, both in the office surgery setting and operating theatre.

Rather than relaxing criteria because of the availability of antibiotics, infection control procedures have assumed increasing importance. In many Australian hospitals we are losing the battle against antibiotic resistant organisms and chronic infections, making the GP office a more attractive environment for minor surgical procedures. Office surgery has increased rapidly as costs of hospitalization have soared.

The isolation of body substance is now recommended, for blood and all body fluids are potentially infectious.

Aseptic technique is practiced not just when the patient is thought to be in a high risk group, for example, a drug user or homosexual, but as a routine.

Key Concepts and Practice Tips.

o Assess all significant skin characteristics in the region before attempting surgery.
o Always consider the functional and cosmetic implications when planning incisions.
o Consider factors which may compromise healing (e.g. vascular disease) or affect the cosmetic result (e.g. keloid tendency) in the particular patient or site.
o All dead tissue and foreign material must be removed from traumatic wounds.
o Remember that superficial appearances may conceal damage to deeper vital structures.
o Assess the site, number and depth of foreign bodies (clinically, by ultrasound and by X-ray if necessary) before attempting removal.
o A foreign body may present as an abscess some time after the initial injury.
o Allow sufficient time to find and remove a foreign body. Ensure approach planned before starting. Consideration needs to be given to attaining a bloodless field by use of a tourniquet.
o A cutaneous lump may be due to a lesion in any layer or appendage of the skin, or in underlying tissues.

o Accurate clinical assessment and diagnosis is vital.
o When operating on a skin lesion, beware of underlying important motor nerves which are superficial at specific sites (e.g. mandibular branch of facial nerve over the body of mandible, the accessory nerve in posterior triangle of neck, and the lateral popliteal nerve over neck of fibula). Motor nerves or sensory nerves may be in danger. Sensory may include nerves such as the supratrochlear, supra orbital or occipital in the forehead and scalp.

Others to be aware of are the post auricular and greater auricular of the ear.

The knowledge of the anatomy of these nerves enables simple nerve block to be performed.

Once again the skin over the ear is taut and painful to infiltrate with local anaesthetic and thus a nerve block may be appropriate.

o Avoid splashing antiseptics into the eyes or on mucosa.
o Alcoholic skin preparations should not be used on sensitive areas of the skin (e.g. scrotum) and can ignite when in contact with diathermy.
o Incisions should be placed in skin creases where they are obvious (particularly in the trunk, neck and face). Avoid incisions which cross joint lines, pressure areas, hair lines and eyebrows.

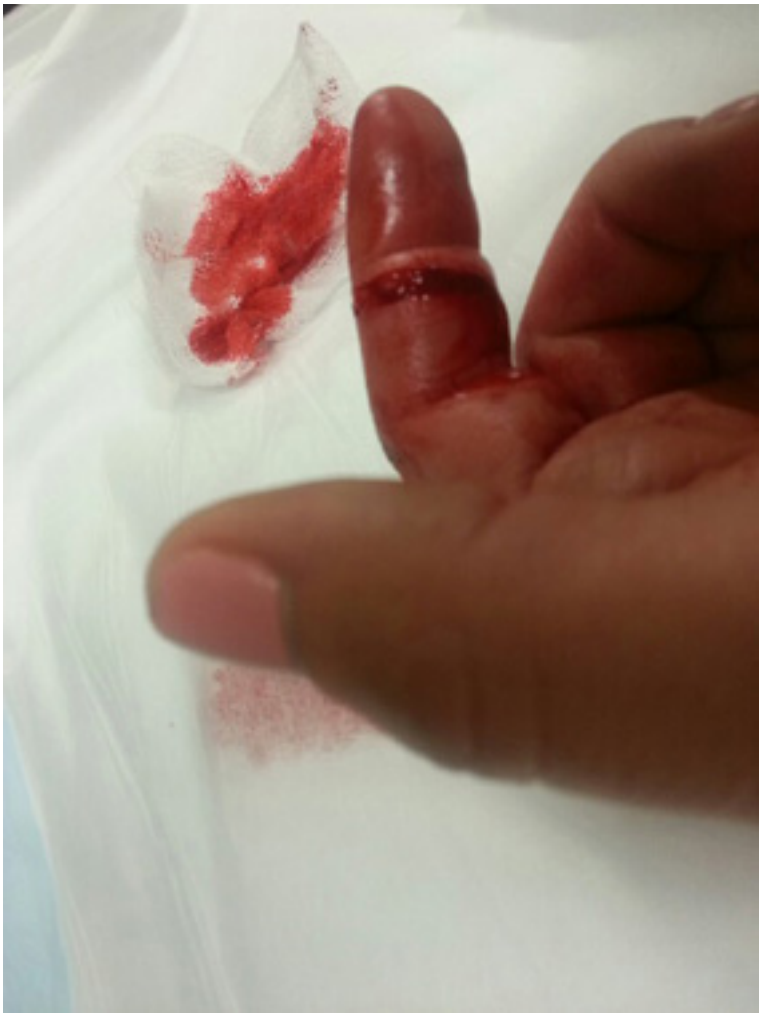
Always align mucocutaneous junctions. The first stitch does this, then follow up with the remainder of the suturing

o Areas with poor blood supply (e.g. skin over the tibia) may not heal well.
o Excess tissue tension impairs blood supply to the wound edge and delays healing.
o Areas with a rich blood supply (e.g. scalp, face and neck) tend to bleed more readily but heal well.
o Beware of factors (e.g. aspirin, anti platelet and anticoagulants or even a family or past history), which may increase bleeding. Surgery may proceed in some cases but the risks need to be considered and explained. Extra care is required. There are some circumstances where these medications can be modified and others where they cannot.
o Excess bleeding may lead to the formation of a haematoma and subsequent infection can cause an abscess.
o Apply pressure as a first measure to control excessive or unexpected bleeding during the procedure..There are other techniques to control bleeding.
o All deep wounds should be closed in layers.
o Avoid leaving a dead space by suturing deeper layers separately or by deep through and through sutures (in trunk).
o Avoid adherence of skin to deeper structures (which can result in a depressed scar) by subcutaneous sutures apposing fascia and muscular layers.
o Fat does not hold sutures well.
o If there is a high risk of infection, delay closure of the wound.
o Interrupted sutures into the skin edge aid haemostasis particularly in vascular areas.
o Everted edges heal better- quicker and with a better scar than inverted edges.
o Mattress sutures help to evert the skin edges preventing inversion which delays healing.
o A subcuticular suture only opposes the epidermis and dermis.
o To some patients the fear of suture removal may be greater than that of the operation.
o Some regions can tolerate some tension to bring edges together. However, in the fingers, lower leg, foot or palm only minimal tension is permissible.
o Err on the side of leaving sutures in place. If there is any doubt, do not remove all of them at the one time. This will prevent wound disruption.
o If sutures appear too tight and causing ischaemia, early removal is advisable.
o Leave sutures for a longer time in areas under greater tension with a poor blood supply or with any other factors which may delay healing.
o A better scar is obtained by using a large number of fine sutures rather than fewer, heavier sutures more widely spread.
o Think of ways to reduce wound tension and swelling (e.g. firm dressings, immobilization, splints).
o Bandages which are too tight impair blood supply.
o Elevation of the limb minimises postoperative swelling.
o Follow up carefully so that postoperative complications can be detected and treated promptly.
o Advise the patient to report urgently any increasing pain, swelling, discolouration, odour or fever.

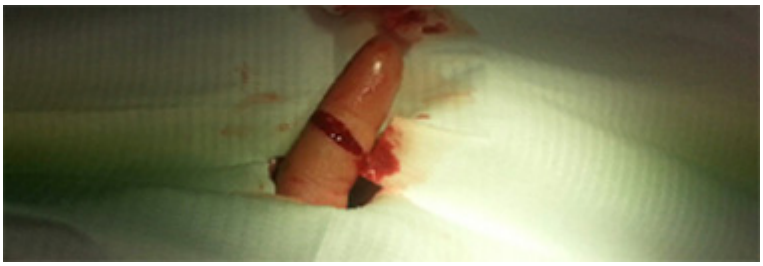
I hope these simple tips will assist you in the office when taking care of your patients and we will continue to present some further tips and visual examples of surgery techniques in the coming series.

Series One - Lacerated Left index finger in 35 year old theatre sister

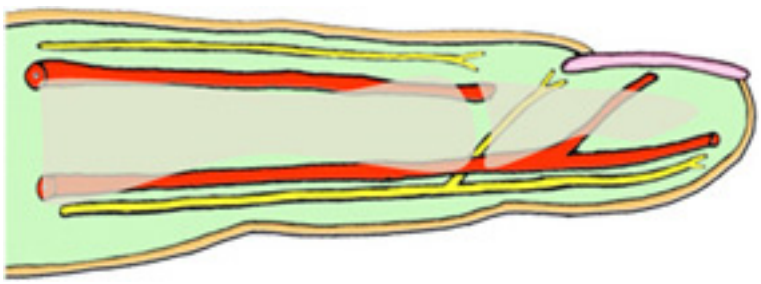
This was a clean incised kitchen wound with a sharp knife. It is on the volar aspect of the index finger.It has been poorly managed due to the basic error of not examining the finger for nerve or tendon injury.



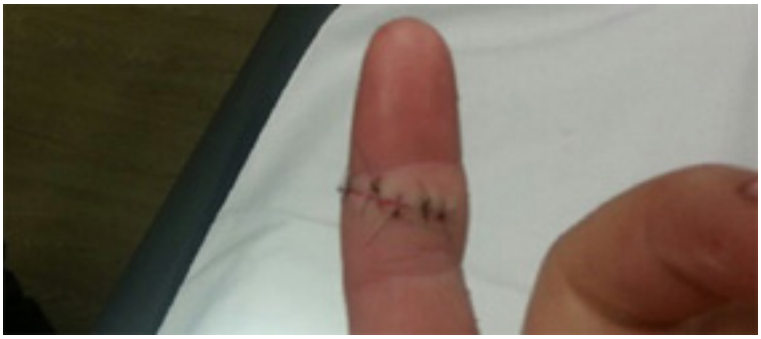
Presenting injury
The patient, a theatre assistant had presented to casualty with a bleeding finger and complaining about numbness. After a long wait local anaesthetic was infiltrated through the wound. The patient had complained that the bleeding was profuse and continued to spurt when pressure was released.



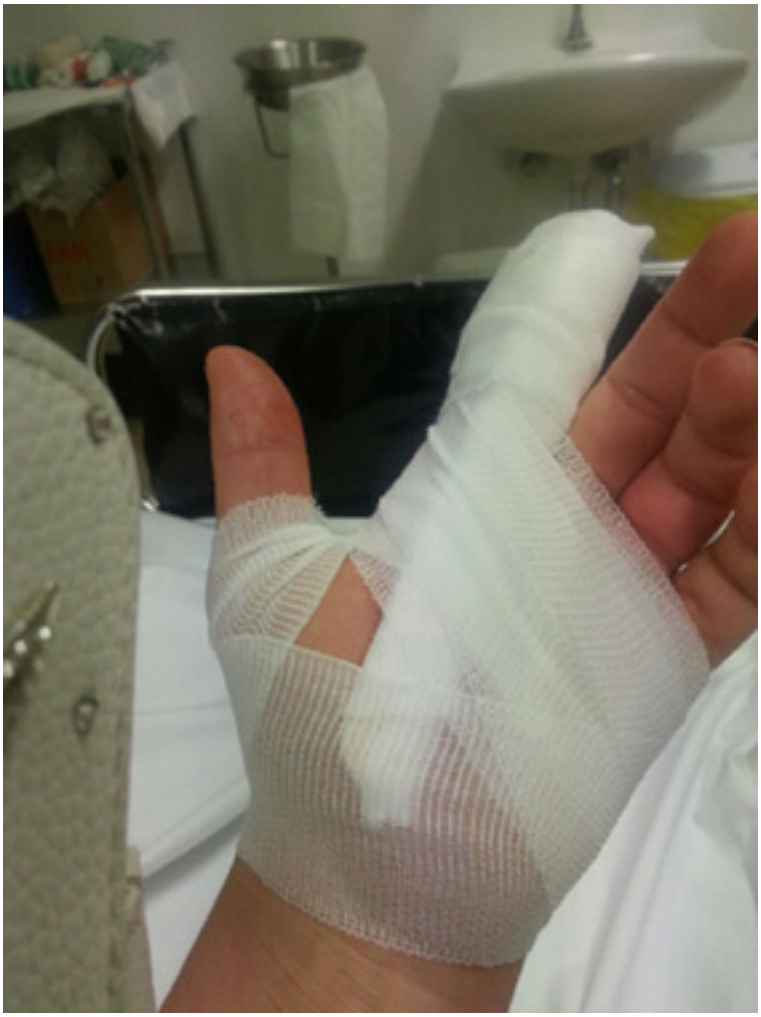
Injury prepared for surgery



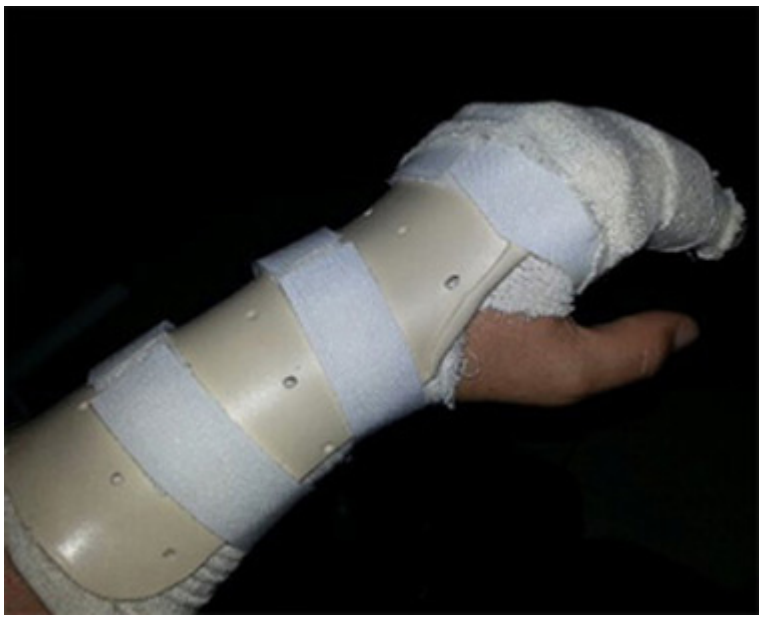
Side view
Arteries and nerves



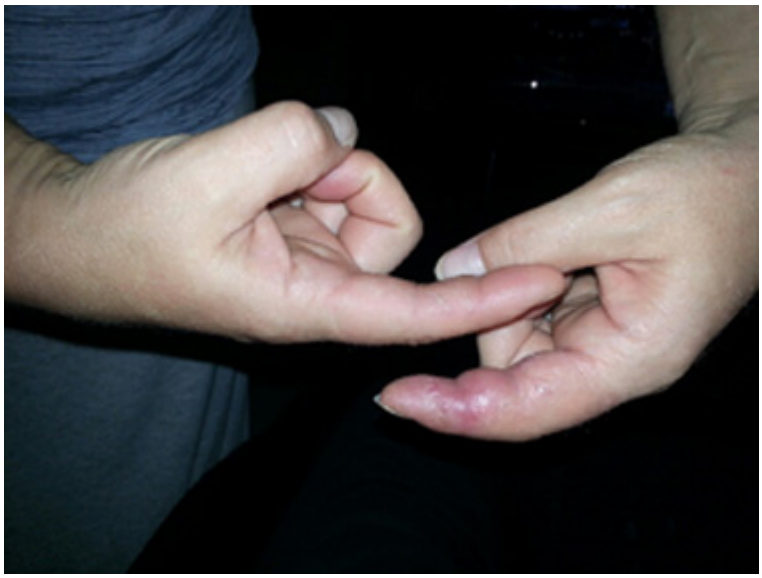
Sutured
The wound was eventually sutured.



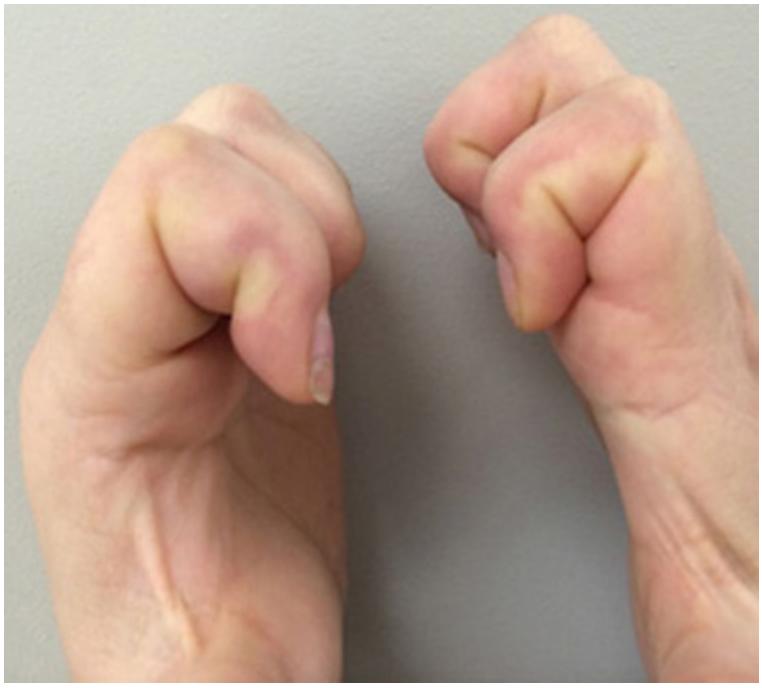
Incorrect splinting
Note that the splinting is incorrect.



Correctly splinted now
The finger is correctly splinted this time in the position of function.This avoids stiffness caused by immobilization.



5 weeks later



Post stiffness

The scrub nurse complained to the surgeon in her theatre 2 days later about the numbness on one side of the finger. This lead to a referral for digital nerve repair. There was also a tendon repair of a partially divided flexor tendon .The skin was sutured this time with a synthetic absorbable 3/0 vicryl suture.Not my preference and it can be seen there is a severe inflammatory reaction with the sutures being spat out intermittently.This occurs more commonly with the multifilamented or braided suture.

The images demonstrate that the initial immobilization was with a straight splint.This was incorrect.This could lead to permanent stiffness.Six months later there is still some restriction on flexion of the finger.

Interestingly :ANATOMICALLY

1- With the fingers and toes the nerves are superficial to the arteries .Thus if an artery is divided (suggested by it spurting) a nerve is likely to have been divided.As well there is a chance there is a tendon injury.

2- Examination must be carried out before the local is used.

3- Whilst local can be injected directly into a laceration it may be advisable to avoid the swelling in fingers to allow better inspection- Thus do a digital block.

4- Splinting of fingers,unless there is a specific reason should be in position of function - not as in this case.This is to avoid stiffness.

5- Some surgeons will use absorbable sutures in the skin - It would not be my choice because of increased reaction with a braided suture.

Absorbable sutures are used in the skin by some surgeons,because they may not need to be removed and thus there is possibly less pain.This also saves the surgeon time.

When we should start writing and publishing an article in the health domain

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Abstract

For novice researchers within the health domain it would be absolutely essential to determine when they should start writing and publishing an article based on their recent research project. There are plenty of reasons which justify writing an article as soon as the necessary data are gathered and analyzed. The aim of the present article is to discuss some of these most important rationales.

Key words: Scientific paper, writing, publishing, health domain

Introduction

Recently the Journal of Clinical Epidemiology (JCE) has published a series of short but fascinating articles on "effective writing and publishing of scientific papers" for "novice academic researchers" (1). The series consists of 12 consecutive papers beginning from: "how to get started" (2) and finishing by: "responding to reviewers" (3). I have found the series very useful especially when I teach novice health researchers in research methodology workshops (4).

My personal experiences also show that for novice researchers it would be absolutely necessary to know when they should start writing and publishing an article based on their recent research project. Unfortunately, evidence suggests that from one hand "time shortages", "continuing study", "problems with co-authors" and "negative results" might totally deter researchers from writing and publishing their results (5). On the other hand however, there are plenty of other vital reasons which justify writing and publishing an article as soon as the necessary data are gathered and analyzed (6). Therefore, the aim of the present article is to discuss some of these most important rationales.

The most important rationales

1. As scientists who work within the health domain it is our responsibility to communicate our new findings as soon as possible (6). Let us imagine that you have new findings regarding the application of a new device or a new drug, etc. It would be absolutely vital to communicate these findings to inform your colleagues and public at large, since your new findings have the potential to save lives or bring amelioration of symptoms in sick people.

2. The second reason which justifies on time publication of new findings is that nowadays different teams of scientists around the world work on rather similar issues. Therefore, if you deliberately delay the publications of your new findings, your colleagues might reach very similar results and publish their findings sooner than you (6). As a result you simply lose your position as a pioneer in your field of expertise.

3. Similar to the previous point, any deliberate delays in publications of new findings put them in danger of becoming old. Nowadays the rate of new research is constantly increasing and we witness huge progress in the health domain, the results of which are published daily. Recently, it has been estimated that more than 5,500 biomedical articles are published on a daily basis (7). Therefore, any intentional long delays in publications would change new findings into old findings.

4. The fourth reason which justifies punctual publication of new findings is that usually editors and reviewers of your manuscript might ask for some revisions. They might even ask for further analyses to be carried out. Therefore, when you write your manuscript as soon as finishing your research project you are fresh enough to carry out more analyses or undertake extra work (6).

5. The final reason which justifies publication of new findings is that you as a biomedical scientist need to promote your career and get funded for your further research. It is only by having a good record on publications especially within the prestigious journals, that you will be able to fulfill this.

Conclusions

For novice researchers within the health domain it would be absolutely essential to realize that they should start writing an article based on their recent research project as soon as possible. The present article provides its reader with five important reasons which justify writing an article as soon as the necessary data are gathered and analyzed. Nonetheless, it is worth emphasizing that on time publications by no means should force scientists to undermine the integrity of research. Similarly, the necessity of having a good record on publications by no means should compel them to breach the ethics of scientific publications (9).

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