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Efficacy of Zinc Supplementation in Reducing the Incidence of Recurrent Vaginitis

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Efficacy of Zinc Supplementation in Reducing the Incidence of Recurrent Vaginitis

By

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ABSTRACT

EFFICACY OF ZINC SUPPLEMENTATION

IN REDUCING THE INCIDENCE OF RECURRENT VAGINITIS

By

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The purpose of this study was to evaluate the efficacy of zinc supplementation in reducing the incidence of recurrent bacterial vaginosis (BV) and vulvovaginal candidiasis (VVC). Forty-one subjects were selected from researcher's patients based on at least two episodes of vaginitis in a six-month period. A control group (placebo) and an experimental group (30 mg zinc gluconate) were randomly formed from this selection.

Evaluation of vaginitis status was accomplished through wet mounts, pH test, and KOH whiff tests at the initial, 3, and 6-month intervals. Subjects sought treatment for vaginitis when symptomatic.

A t-test analysis showed no statistically significant difference in the overall incidence of vaginitis between the control group and the experimental group ($t = 1.45$, $df = 22$, $p = 0.16$). The incidence of VVC in the zinc group decreased from 19 to 5 post treatment ($t = 2.23$, $p = 0.021$).

DEDICATION

This work is dedicated to Jim, Rich and Carl.

With gratitude for your faith, support, and encouragement.

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I wish to acknowledge and thank the members of my committee:

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CHAPTER 1

INTRODUCTION

Vaginitis accounts for more visits to the obstetrics and gynecology office than any other female condition (Mead & Eschenbach, 1998). An estimated 5 - 10 million office visits per year are made due to vaginitis (Faro, 1994). In 1991, U.S. women spent over $100 million dollars on over the counter yeast treatments (Foxman, 1997).

Bacterial vaginosis (BV) is the most common vaginal infection. It is estimated that 50 - 64% of sexually active women have at least one episode of BV (Selleck, 1997). Vulvovaginal candidiasis (yeast infections or VVC) are the second most common vaginal infection. An estimated 75% of women will have a VVC infection at least once in their lives (Nyirjesy, 1997). Approximately 5% of women with one episode of VVC will suffer difficult or recurrent infection. Recurrent infection is commonly defined as four mycologically proven symptomatic episodes of VVC in 12 months (Nyirjesy, 1997).

Recent research links BV to serious gynecological, obstetrical, and general medical risks, including upper reproductive tract and postoperative infections, adverse pregnancy outcomes, preterm labor, cervical dysplasia, mucosal barrier disruption, and increased transmission of Human Immunodeficiency Virus (HIV), Human Papilloma Virus (HPV), and Herpes Simplex Virus (HSV) (Secor, 1997). Little research has been done in finding nursing interventions that may help decrease the recurrence rate of vaginitis in women who are prone to recurrent infections.

Health care professionals have long known that zinc is needed by the human body for optimal well-being, especially for maintaining a healthy immune system. The use of topical zinc has been recommended in the treatment of genital herpes (Northrup, 1994).

High levels of serum zinc have been observed as a protective factor against HPV in women (Liu, Soong, Alvarez, & Butterworth, 1995) and HIV (Secor, 1997). Zinc has also been found to have an antitrichomonal activity (Houang, Ahmet, & Lawrence, 1996). Zinc deficiency has been demonstrated to reduce the immunity of inbred strains of mice to infection with *C. albicans* (yeast), while zinc excess or sufficiency was a protective factor against Candida (Edman, Sobel, & Taylor, 1986).

This author identified no studies on the use of supplemental zinc to help prevent vaginal infections. "Research that describes the microfloral ecosystem of the vagina or examines therapeutic or preventive modalities based upon ecologic tenets is sparse" (Overman, 1993, p. 146). "The scientific literature increasingly supports the impact of lifestyle on health maintenance and disease prevention; yet, there are few scientific data to support the role of nutrition, exercise, stress reduction, and general health maintenance in the management of sexually transmitted diseases (STDs) and vaginitis" (Roe & Gudi, 1997, p. 275).

Many patients seek a natural remedy for health care ailments. "In a given year about a third of all American adults use unconventional medical treatments. The most frequent users are educated, upper income White Americans in the 25 to 49 year age range" (Youngkin & Israel, 1996, p. 39). Because patients are seeking newer, unconventional treatments for common health care concerns, nurses must embrace the study of safe, complementary and alternative treatment methods. Therefore, the purpose of this study was to determine if the use of supplemental zinc would decrease the incidence of vaginitis in women prone to recurrent infection.

CHAPTER 2

CONCEPTUAL FRAMEWORK AND LITERATURE REVIEW

Conceptual Framework

Nursing Theory - Neuman's Systems Model

Basic theory. The Neuman's Systems Model (1993) was the theoretical framework for this thesis. The model can best be explained by using a diagram consisting of several concentric circles (see Figure 1). The inner circle is the "basic structure" and represents the individual, family, or a system. The basic structure has several common factors including: normal temperature range, genetic structure, response pattern, organ strength or weakness, and knowns or commonalties (a common range of normal characteristics and responses).

The next in the series of circles are the "lines of resistance". These lines represent the body's defenses that are involuntarily activated when a stressor invades the normal line of defense. They attempt to stabilize the client to return to the normal line of defense (Neuman, 1993).

The "normal line of defense" is the next outer circle. It represents the normal wellness level, or what the client has become. It is a result of the client's previous interactions with environmental stressors, or more simply, her coping skills. It is the standard from which deviances from wellness can be judged (Neuman, 1993).

The outermost concentric circle is the "flexible line of defense". It represents a protective buffer for the client's normal state. It serves to prevent an invasion of stressors.

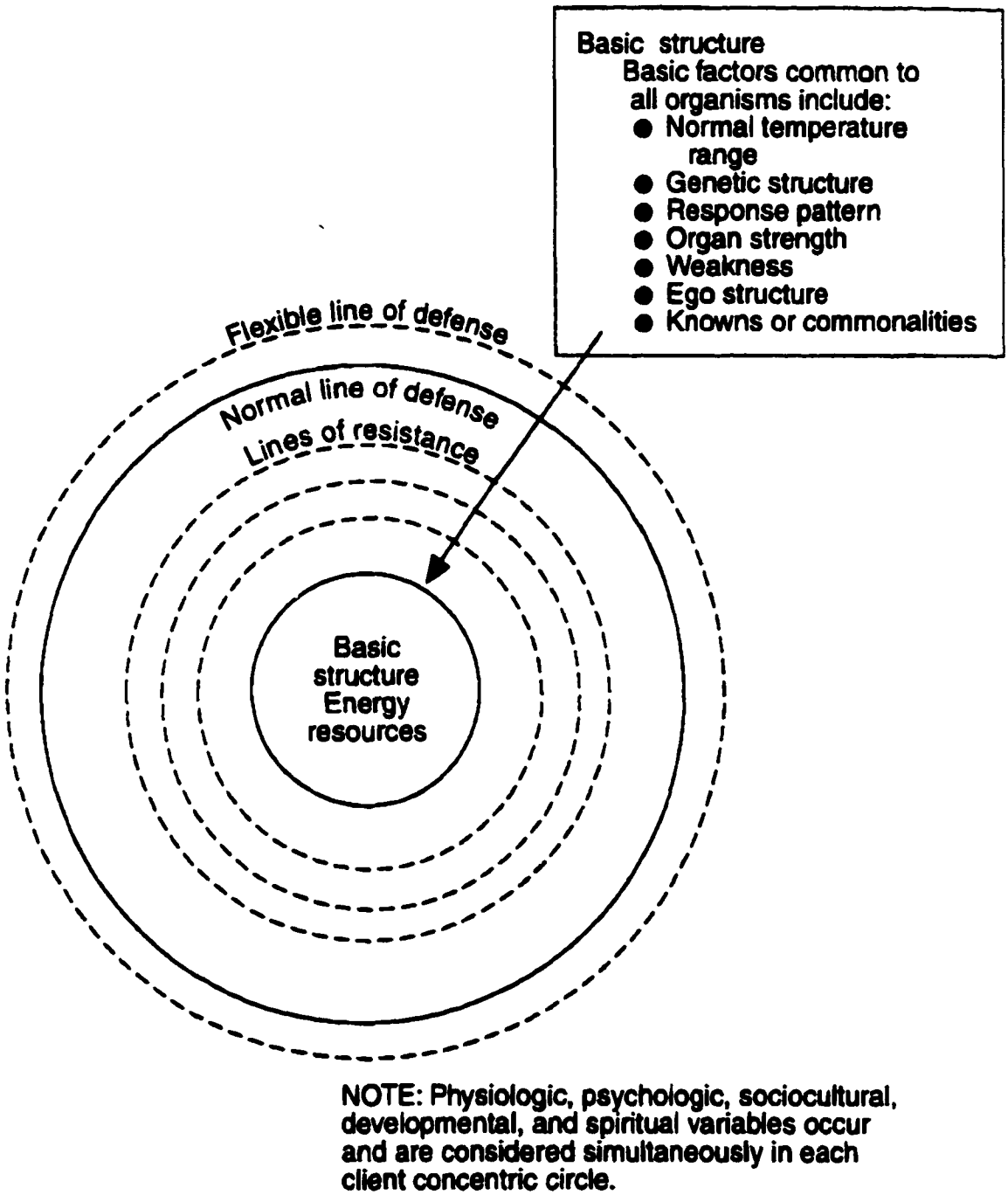

Figure 1. Diagram of the Client System. From "The Neuman Systems Model," by B. Neuman, 1989, p. 28. Copyright 1989 by Appleton & Lange. Used with permission of the author.

This line can be thought of as accordion-like. The further away it is from the normal line of defense, the stronger it is. This line varies easily and is affected by nutrition, exercise, mental, physical, and spiritual health (Neuman, 1993). Definitions of Neuman's paradigm concepts are listed below. Application of each concept to the proposed study is clarified.

Concepts. Neuman defines her paradigm concepts as:

1. Person - a composite of five variables: Physiologic, psychologic, sociocultural, developmental, and spiritual. Zinc supplementation is proposed to affect the physiologic variable.

2. Environment - has internal and external components. This can be thought of as the vaginal ecosystem.

3. Health - Varying degrees of harmony and balance between internal and external environment through a process of interaction and adjustment. Zinc may affect the balance of health by strengthening natural killer cell activity in the vagina.

4. Nursing - Primary goal is retention, attainment, or maintenance of client stability (Fawcett, 1995). The goal for nursing could be the vagina's ecological or physiological stability.

Assumptions. Neuman outlined ten basic assumptions inherent in the Neuman Systems Model (Fawcett, 1995). For the purpose of this paper, the following will be defined:

1. Though each client is unique, each system is a composite of common known factors or innate characteristics within a normal, given range of responses, contained within a basic structure.

2. Many known environmental stressors exist. Each differs in its potential for disturbing a client's usual stability level, or normal line of defense. Particular client variables (in this study the physiologic variable), can affect the degree to which a client is protected by the flexible line of defense against a possible reaction to a stressor.

3. Each client, over time, has evolved a normal line of defense. This line varies for each individual. Some people are more resistant than others.

4. When the cushioning accordion-like effect of the flexible line of defense is no longer capable of protecting the client against an environmental stressor, the stressor breaks through the normal line of defense. The interrelationships of variables - physiological, psychological, sociocultural, developmental, and spiritual - determine the nature and degree of the system reaction to the stressor.

5. The basic structure is a dynamic composite of the interrelationships of the five variables. Wellness moves on a continuum of available energy to support the system in its optimal state.

6. Implicit within each client system is a set of internal resistance factors known as lines of resistance, which function to stabilize and return the client to the usual wellness level (normal line of defense).

7. Primary prevention relates to general knowledge that is applied in client intervention and reduction of risk factors associated with stressors to prevent possible reaction. This would include client education for basic vaginal hygiene.

8. Secondary prevention relates to symptomatology following a reaction to stressors, and treatment to reduce their noxious effects. This would include treatment of a vaginal infection.

The Neuman's System Model has a unique focus on the wellness of the client in relation to environmental stress and reactions to stress (Fawcett, 1995). It allows easy conceptualization of how optimal nutrition, including zinc supplementation, could strengthen the body's resistance to recurrent vaginal infections.

Vaginitis Theory

To conceptualize how zinc may affect the vagina's resistance to infection, the author developed a diagram of the vagina and its ecosystem patterned after Neuman's Model (1989) (see Figure 2). In the author's diagram, Neuman's basic structure represents the

vagina, which includes a number of basic factors including normal temperature range, genetic structure, response pattern (to stressors), and organ strength or weakness (skin integrity, general health of woman).

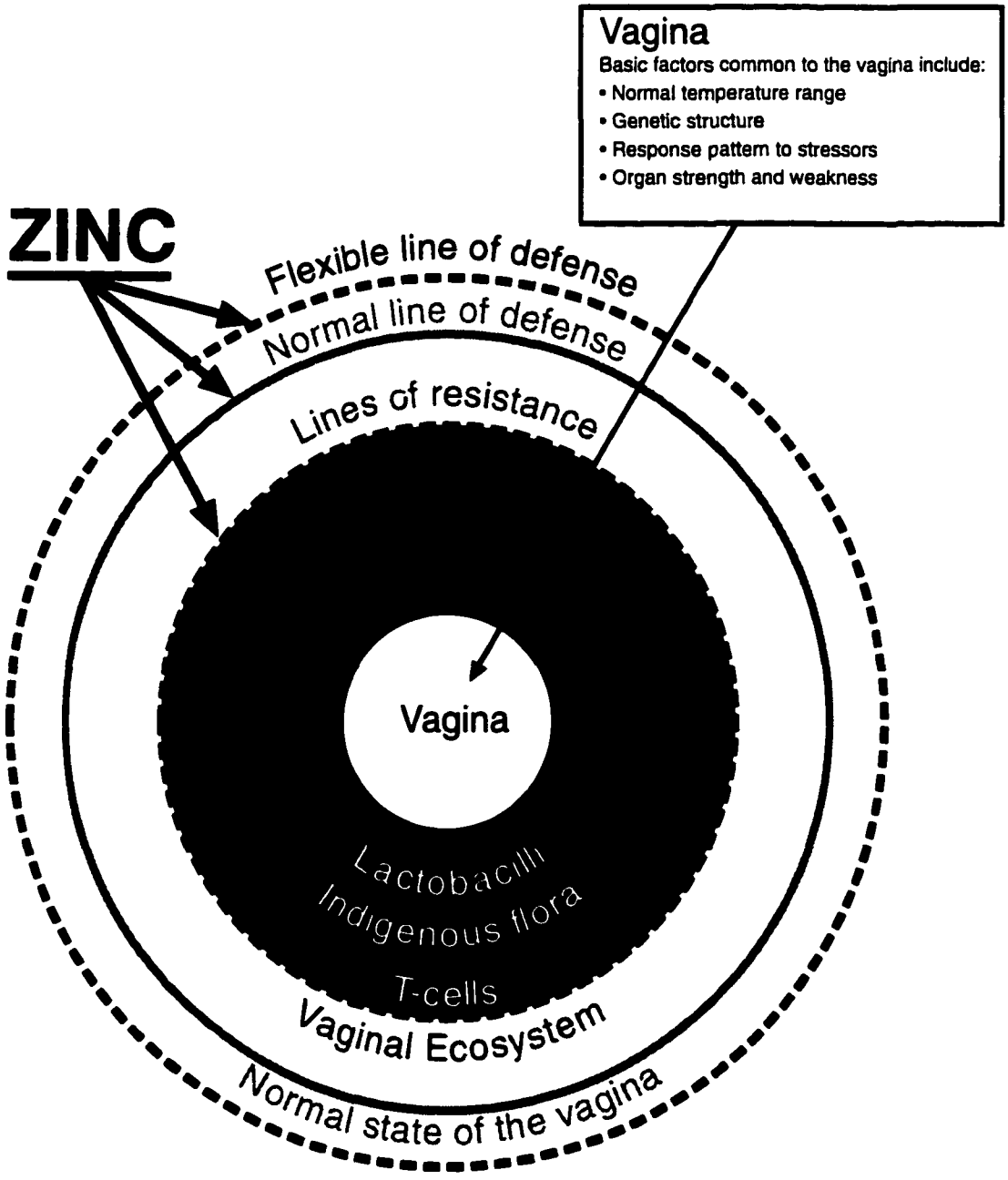

Figure 2. Vaginal Ecosystem Based on Neuman's Systems Model

Neuman's lines of resistance represent the vaginal ecosystem (vaginal microecology system). The vaginal ecosystem contains many microorganisms that stabilize the vagina and attempt to return the vagina to its normal state. Lactobacilli is the dominant organism present in the normal vaginal ecosystem. These lactobacilli produce hydrogen peroxide which keeps the vaginal pH acidic and prevents overgrowth of bacterial pathogens (Sobel, Cook, & Redondo-Lopez, 1991). Lactobacillus acidophilus is thought to maintain ecological control in the vagina by competing with other organisms for adherence to the vaginal wall, producing protein inhibitors with antimicrobial properties and stimulating the immune system (ReDondo-Lopez, Cook, & Sobel, 1990).

When the vaginal ecosystem is disrupted either by a change in the vaginal flora or a change in the host tissue, vaginitis can occur (Overman, 1993). Changes in the normal flora can be due to age, hormone status, excessive douching, pregnancy, influence of drugs (especially antibiotics), intrauterine device use, frequent intercourse, new sex partner, or a compromised immune status (Kent, 1995; Ament & Whalen, 1996; Star, 1995; Tobin, 1995). When the normal flora are altered, pathogenic bacteria flourish (Kent, 1995). T cells also exist in the vaginal vault and are responsible for attacking foreign organisms. A logical conclusion then is that maintaining a healthy vaginal ecosystem is an important factor in preventing vaginal infections.

Changes in the vaginal ecosystem can also be due to stress. Stress results in large amounts of both adrenal and glucocorticoids being released into the blood stream (Cataldo, DeBruyne, & Whitney, 1995). "One of the most significant effects of stress-induced secretion of glucocorticoids is seen in immunologic function. Excessive glucocorticoids produce anti-inflammatory effects. Glucocorticoids also inhibit the release of histamine and other kinnins, thus limiting the physiologic cascade of events involving the inflammatory response. The result is anti-inflammation and increased susceptibility to infection" (Sikes, 1992, p. 385).

The normal line of defense represents the normal state of the vagina. This is affected by the general health and well being of the woman. For instance diabetes, AIDs, autoimmune disorders, and a high-stress lifestyle can make a woman more prone to recurrent vaginal infections.

The flexible line of defense is a protective buffer and acts to prevent invasion of stressors. The flexible lines of defense are the interrelationships of physiological, psychological, sociocultural, developmental, and spiritual variables. These variables determine how susceptible a woman will be to stressors and also affect the balance of the vaginal ecosystem. Because this line is affected by nutrition (physiologic variable), zinc supplementation may strengthen the flexible line of defense.

In summary, Neuman's Model (1989) and the author's vaginitis theory are comprised of 4 components. For the purpose of this study, Neuman's basic structure represents the vagina. Neuman's lines of resistance represent the vaginal microecology or vaginal ecosystem. Neuman's normal lines of defense are perceived as the normal state of the vagina. Neuman's flexible lines of defense remain unchanged as the interrelationship of the five variables stated above.

Review of Literature

This section reviews several studies that have linked zinc deficiency to recurrent vaginal infections. The studies have focused on candidiasis, trichomoniasis, and Human Papillomavirus. The candidiasis study measured plasma and tissue concentrations of zinc, while the trichomoniasis study focused on treatment with zinc douches. Several studies have been done on the use of topical zinc in wound healing that suggest positive results. Zinc oxide has long been used as an over the counter treatment for and prevention of diaper rash, athlete's foot, and sunburn. The researcher has not found any studies involving oral zinc supplementation as a prevention modality for vaginitis.

Zinc

Zinc properties. The mineral, zinc, plays an important role in the formation of protein in the body and thus assists in wound healing, blood formation, and general growth and maintenance of all tissues. Zinc is a component in many enzymes and is, thusly involved in most metabolic processes (USDA, 1990). Another property of zinc, which has been hypothesized to play a role in normal physiological function, is zinc's capacity to stabilize membranes. This effect has been observed where zinc protects against various inducers of hemolysis such as bacterial toxins (Walsh, Sandstead, Prasad, Newberne, & Fraker, 1994).

Delayed wound healing is a sign of moderate zinc deficiency. Recent studies show that a mild or marginal deficiency of zinc in humans is characterized by decreased natural killer cell activity and alterations in T cell subpopulations (Walsh et al.).

Zinc is a relatively safe mineral. Doses up to 6 times the Recommended Daily Allowance (RDA) are considered nontoxic in the healthy individual (Walsh et al.). Taking more than 100 mg a day of zinc for extended periods of time could result in problems such as depressed immune function and imbalances of copper. The use of high doses of zinc for longer than seven days at a time is not recommended (Herb Research Foundation, 1997).

The US RDA of zinc is 15 mg for males, 12 mg for females, and 15 mg for pregnant women (Walsh et al.). Studies show that many US adults get only 9.9 mg a day, and typically women average only 8.1 mg per day (American Zinc Association, 1997).

The cost of oral zinc supplements averages $5.00 for 100 capsules (30 to 50 mg) or $1.50 per month, significantly less than the average $15 for over the counter VVC treatments. A rare side effect is mild gastric distress. Taking the capsule with food can alleviate this.

Zinc toxicity. The average US citizen is at far greater risk of becoming zinc deficient than experiencing adverse health effects as a result of exposure to high zinc levels. Determining what effects excess zinc has on immune function is more problematic because

exposure to such levels is rare, and available literature is limited (Walsh et al.). A 1984 study suggests evidence that excessive zinc intake is toxic to the immune system. In this study, 11 adult males were given 300 mg of elemental zinc daily for 6 weeks. This level is 20 times the RDA. This study is considered to have several flaws. Because high levels of zinc intake can cause copper deficiency, a major flaw in this study was the failure to assess serum copper levels in the study subjects. Another flaw concerns the measures used to assess immune status. Results of this study showed a depressed proliferative response of lymphocytes, yet no change in the absolute number of lymphocytes and T and B cells. Also noted was a reduction in the ability of phagocytic bacteria (Walsh et al.).

A second study involved giving elderly subjects 440 mg of zinc sulfate a day for one month. This study found that high levels of zinc intake had no adverse immunological or physiological effects on the elderly (Walsh et al.).

The major consequence of long-term ingestion of excessive zinc supplements is induction of secondary copper deficiency caused by competition between these elements for intestinal absorption. Levels of zinc supplements as low as 45 mg per day have negatively influenced copper status (Severus, Oepen, & Stoll, 1996). Long term zinc supplementation in excess of 150 mg per day has also been reported to result in low high density lipoprotein (HDL) levels, gastric erosion, and depressed immune function (King & Keen, 1999). Chronic zinc toxicity is characterized by gastric problems, hypocupremia, and reduced HDL cholesterol (The Herb Research Foundation, 1997).

"Acute, high level exposure to zinc compounds can produce respiratory and gastrointestinal toxicity. However, these effects are largely self-limiting and require only modest medical attention." (Walsh et al., p. 34). Most exposures at these levels have been through inhalation of smoke bombs or chronic exposure to fumes that are byproducts of welding. Signs of acute zinc toxicity include: gastric distress, dizziness, and nausea.

Calcium and zinc absorption. Calcium supplementation at levels of 1500 mg per day has been shown to decrease zinc absorption (Tufts University Health & Nutrition

Letter, 1997). In 1997, Wood and Zheng investigated the effects of calcium supplementation on zinc absorption and balance in 18 relatively healthy, postmenopausal women aged 59-86 years. All subjects received a standardized diet of typical foods supplying 17.6 mg of zinc, and 890 mg of calcium during a 36 day period. The zinc balance study was divided into three, 12 day experimental periods. In two of the three cycles an additional 468 mg of calcium was added to the participant's diet. Net zinc absorption and zinc balance were significantly reduced by approximately 2 mg per day during both high calcium treatment periods ($p < 0.05$). Measurements were obtained from urine samples and fecal specimens. The researchers stated that "The mechanism through which high calcium diets influence net zinc absorption is poorly understood" (Wood & Zheng, 1997, p. 1808).

Zinc and the common cold. Several studies have been done using oral zinc gluconate lozenges (23 mg) to reduce the length and severity of common cold symptoms. At least eight studies have been performed. Of these studies, four found that zinc supplementation was beneficial, and four found that it was not (Jackson, Peterson, & Lesho 1997). The average dose of zinc gluconate supplementation in those studies was 23 mg every 2 hours while awake for 7 - 10 days. That is an average of 184 mg daily.

Immunopathology of zinc deficiency. Studies on dietary zinc deficiency on the immune function of rodents represents the best developed paradigm regarding the effects of a nutritional deficiency on host defense systems. These studies indicate that zinc is essential to maintaining the integrity of the immune system (Walsh et al.). Information gathered to date indicates that a 30 day period of suboptimal intake of zinc in the young adult mouse, which produced a 20 to 25% weight loss, reduced the thymus to a quarter normal size and depleted the lymphocytes and macrophages in the spleen 50 - 75% (Walsh et al.). The thymus gland has a profound influence on overall immune system response in individual cells and blood and lymph (Scheer, 1998). Antibody mediated responses to both T-cell dependent and T-cell independent antigens were reduced 50 to 70%. Delayed-type

hypersensitivity skin reactions, and the function of natural killer cells were also significantly reduced in zinc deficient mice (Walsh et al.). Antibody mediated responses are key to immune defense against a variety of pathogens, so such reductions explain the increased incidence of infection observed in zinc-deficient subjects (Walsh et al.).

Zinc deficiency can lead to many complications, including reduced immune function (American Zinc Association Newsletter, 1997). "Zinc deficiency is associated with depression of a number of cellular immune functions, including delayed-type hypersensitivity reactions, T-helper cell activity, development of cytotoxic T cells, natural killer cell activity, and mitogen-induced T-lymphocyte proliferation" (Edman, Sobel, & Taylor, 1986, p. 1084).

Edman et al. (1986) state that zinc deficiency has been demonstrated to reduce the immunity of inbred strains of mice to infection with *C. albicans*, while zinc excess or sufficiency was a protective factor against *Candida*. In theory then, correcting a zinc deficiency would help maintain a normal colonization of lactobacilli in the vagina.

Zinc deficiencies result in decreased antibodies and lymphocytes, leading to impaired body fluids and cell-mediated immunity, as well as an increased susceptibility to a variety of infections (Scheer, 1998). Deficient zinc intake can profoundly depress both T and B cell function. Depression of B and T cell formation is manifested as a progressive increase in infections with opportunistic micro-organisms especially *Candida albicans* and other fungi (Walsh et al.). Zinc is required as a cofactor for at least 70 different enzymes, some of which are found in lymphocytes and are necessary for their function. Several immunologic factors have been proposed to be associated with recurrent VVC. Women with recurrent VVC have been found to have reduced antigen-specific-T-lymphocyte reactivity (Nyirjesy, 1997).

Vaginitis

The pathophysiology of vaginal infections, both *candida* (yeast) and bacterial, has long been recognized as the disruption in the normal vaginal flora. The vaginal environment

is a dynamic and a delicate ecosystem (Mardh, 1991). Normal vaginal pH is 3.8 - 4.2. Normal vaginal flora consists of yeast, bacteria, and hydrogen-peroxide producing lactobacilli. Lactobacilli, as stated earlier, are thought to maintain ecologic control of the vaginal ecosystem.

Bacterial vaginosis. BV is a major disruption in vaginal flora characterized by a massive overgrowth of vaginal anaerobes, and the disappearance of Lactobacillus species, especially those that produce hydrogen peroxide (Sobel, 1997). The relative lack of normally occurring lactobacilli alters the ecosystem of the vagina (or the lines of resistance), allowing anaerobes to predominate and producing metabolic by-products that raise the pH level of the vagina. Research to date has not clarified the cause of the microecologic shift resulting in BV (Overman, 1993). Amines, the end products of anaerobic metabolism, produce a fishy smelling discharge that is the hallmark of BV (Star, 1995).

Vulvovaginal candidiasis. Yeast infection or vulvovaginal candidiasis (VVC) is an upset in the homeostatic balance (normal line of defense) in the vagina that leads to an overgrowth of the fungus yeast (*Candida*). Three main types of *candida* affect the vaginal tract: *candida albicans, candida tropicalis, and candida glabrata. Candida albicans* is the most common organism found. Over the counter yeast treatments kill only *candida albicans*, not the lesser found *candida* organisms. The yeast organism gains access to the vaginal mucosa primarily from the perianal area (Youngkin & Davis, 1994). The warm, moist environment of the vulva and vagina, in combination with its close proximity to the rectum, make it a favorable reservoir for colonization of the yeast fungus (Secor, 1997). *Candida albicans* is found in the normal (asymptomatic) vaginal flora of premenopausal non-pregnant women with a 11% prevalence rate (Secor). The hallmark symptom of VVC is vulvar pruritis (Foxman, 1997).

Zinc and vaginitis. Several studies have been done using zinc treatments for the diagnosis of VVC, trichomoniasis, or HPV. The author did not find any studies relevant to zinc and BV.

The first study reviewed evaluated the effects of potential risk factors such as HPV 16 and nutritional status on the course of cervical dysplasia. Liu, Soong. Alvarez, & Butterworth (1995) found that women who were HPV 16 positive and had high levels of plasma zinc were less likely to develop cervical dysplasia than those without high levels of plasma zinc were . The study involved 206 women in Birmingham, Alabama. With the use of data of four repeat evaluations of dysplasia at an interval of 2 months, specific relationships between HPV-16 infection, plasma retinol and zinc levels, and dysplasia progression were evaluated through longitudinal data analysis of generalized estimating equations.

Andrews, Mylvaganam, and Yule, physicians from Norway and the United Kingdom, undertook a study in 1994 involving resistant strains of *Trichomonas vaginalis, Tritrichomonas foetus*, and *Giardia intestinalis.* The activity of bacitracin with and without the addition of zinc on the resistant strains was studied using isolates in aerobic and anaerobic conditions on culture plates. Minimum Lethal Concentrations of bacitracin alone against *Trichomonas vaginalis* were 1500 - 2500 iu/mL. By adding zinc to the bacitracin the Minimum Lethal Concentrations needed to destroy *T. vaginalis* dropped to 250 - 400 iu/mL. The primary action of bacitracin zinc is thought to be directed at the bacterial cell wall. The activity of bacitracin against Trichomonas was enhanced 5 - 10 times with the addition of zinc. This was true in both aerobic and anaerobic culture conditions and was equally effective against isolates that were sensitive or insensitive to metronidiazole (Andrews, Mylvaganam, & Yule, 1994).

The next study reviewed involved four patients with a 4 month to 4 year history of culture-positive symptomatic trichomoniasis who had received a variety of therapies before referral. The patients selected had documented evidence of recurrent symptomatic trichomoniasis unresponsive to repeated courses of conventional therapy (metronidiazole). Another criteria for inclusion in the study was evidence that the chronic recurrence was not due to reinfection from an infected partner. The patients were instructed to douche with 25

mL of zinc sulfate solution in 250 mL of distilled water. After douching, they inserted a 500 mg metronidiazole suppository vaginally. This therapy was given twice a day for the first week, and then gradually tapered off over 5 months. At a review 2 to 5 months after therapy, all patients remained asymptomatic and culture negative (Houang, Ahmet, & Lawrence, 1997). The exact role of zinc sulfate douching is not known, but the combination of zinc and metronidiazole therapy achieved a cure in these patients at a dosage that had failed before, when metronidiazole was used alone (Houang, et al., 1997).

Another case study involved a woman with trichomoniasis who failed to respond to high dose metronidiazole, over a 4 month time frame. At completion of this therapy she was still found to be culture positive. She was admitted for intravenous (IV) metronidiazole therapy, 500 mg three times a day for 5 days. Within one week of IV therapy, urethral and vaginal cultures were positive for trichomoniasis. At this stage, plasma and urinary zinc levels were measured. Her plasma zinc level was 9 (normal 12 - 20), and urinary zinc level was 3 (normal 4 - 12). She was prescribed zinc sulfate, 220 mg orally twice a day for 3 weeks. Her plasma zinc level rose to 12. After 15 months of recurrent infection, this patient was culture negative for four months after treating her low plasma zinc level (Willmott, Say, Downey, & Hookham, 1983).

A specific study on zinc and candidiasis took place in 1985. Drs. Edman, Sobel, and Taylor evaluated the relationship between zinc status and recurrent vaginal candidiasis. Twenty-nine subjects with at least 3 documented episodes of acute *Candida* vaginitis in the previous 12 month period comprised the experimental group. The control group consisted of 20 women who had never been diagnosed with vaginal candidiasis. The two groups were matched for age, race, and parity. None of the subjects were pregnant, postmenopausal, or had any chronic health conditions. Seven subjects with recurrent vaginitis (24.1%) were taking zinc-containing supplements, while five control subjects (25%) were taking zinc-containing supplements. Dietary zinc intake was assessed based on 3 day food

consumption records. The results suggested that dietary zinc intake between the two groups was not statistically significantly different ($p = 0.190$).

Plasma zinc measurements were then obtained from both groups. The mean plasma zinc concentration in the recurrent vaginal candidiasis group was 87 μg/dl in zinc-supplemented women and 79 μg/dl in non-zinc-supplemented women. In the control group, mean plasma zinc levels were 95 μg/dl in the zinc-supplemented women, and 91 μg/dl in the non-zinc-supplemented women. The mean plasma zinc concentration was significantly lower in women with recurrent vaginitis not receiving zinc supplements (79 μg/dl) when compared with control nonsupplemented women (91 μg/dl.) ($p = 0.015$). Comparison of the women in both groups who were receiving zinc supplements showed the plasma concentration was lower in women with recurrent infection (87μg/dl), than in the control group (95 μg/dl), but this was not statistically significant ($p = 0.52$) (Edman et al., 1986).

The results of this study suggest that women in the group not receiving zinc with recurrent vaginal candidiasis had significantly lower mean plasma zinc levels than women who had never had an episode of *candida* vaginitis. Limitations of this study include the fact that the zinc supplements were not standardized. There is no indication of how many milligrams of zinc the women were taking or whether it was the same type of zinc (i.e., zinc gluconate, zinc acetate, etc.) (Edman et al.).

Edman et al. suggest that "additional studies are required to correlate zinc status in women with recurrent vaginal candidiasis. Controlled longitudinal studies are necessary to correlate the effects of zinc supplementation with immune status in susceptible women and to evaluate the effects of zinc supplementation on the clinical recurrence rate of *candida* vaginal infection. In light of the available data correlating acquired zinc deficiency with reduced T-lymphocyte function, a parameter known to be impaired in women with recurrent vaginal candidiasis, zinc deficiency may be a contributing factor in some women with recurrent vaginal candidiasis" (1986, p. 1085).

Following publication of the previously reviewed study, Bohler, Meisinger, Klade and Reinthaller (1994) undertook a study measuring zinc levels of serum and cervicovaginal secretions in women with recurrent VVC. Twenty-one women with at least three documented episodes of VVC within the previous 12 months, and 15 women without VVC as a control group were investigated.

Results showed no significant difference in the mean plasma zinc concentrations between the recurrent VVC group (109 μg/dl) and the control group (107 μg/dl). Measurements of cervicovaginal secretions showed 30.2 μg/dl of zinc in the recurrent VVC group, and 29.6 μg/dl of zinc in the control group. This difference was not significant. (No normal zinc values of cervicovaginal secretions have been established). While no confirmation of zinc deficiency in women prone to recurrent VVC was found, "controlled studies should help to clarify whether zinc supplementation affects clinical cure rate of VVC" (p. 310).

This study is limited by that fact that dietary zinc intake was not assessed. Also, it is not known whether any of the subjects were using zinc supplementation. It is also not known whether zinc levels in cervicovaginal secretions are a valid measurement in regards to this topic (Bohler et al.).

Summary and Implications for Study

The vaginal environment has an innate immune system (lines of resistance). Changes in the immune system, which disturb the delicate balance of lactobacilli, can result in vaginitis. Zinc is known to enhance the immune system. Conversely, zinc deficiency can negatively impact the vaginal immune system. Correcting a zinc deficiency, by a safe, cost-effective method may decrease the incidence of recurrent vaginitis.

The literature points to several instances in which the use of zinc enhanced treatment of resistant vaginitis. Research suggests that zinc supplementation can help the body's immune system response to infection. There is little data or knowledge available to nurses, physicians, and the general public about what preventive measures can be taken to reduce

the incidence of recurrent vaginitis. Zinc supplementation alone has never been studied in relation to prevention or treatment of recurrent vaginal infections. Nursing could certainly benefit from this study if a positive correlation is shown. Although this was a small study, it provides a starting point for a more comprehensive research study in the future.

Purpose/Hypothesis

Maintenance of a healthy vaginal microecology is vital in the prevention of vaginitis. Because zinc deficiencies can lead to impaired immune functioning, correcting a presumed zinc deficiency may help maintain a normal colonization of lactobacilli in the vagina, thereby reducing the incidence of recurrent vaginitis. The purpose of this study was to evaluate the effect of oral zinc supplementation on the recurrence of vaginitis (either VVC or BV) in women ages 18 - 45 that are prone to recurrent vaginitis.

The research hypothesis stated women, between the ages of 18 - 45 with recurrent vaginitis who take 30 mg of oral zinc supplementation, will have fewer cases of vaginitis than women who do not supplement during a six month time frame.

Definition of Terms

Recurrent vaginitis is defined as two or more episodes of clinician-identified vaginitis in a 6 month time frame.

Oral zinc supplementation for this study is defined as 30 mg of zinc gluconate daily in capsule form.

Presence of clinician diagnosed vaginitis was evaluated during pre-determined study intervals and/or as a result of patient request. Evaluation included a vaginal pH, a microscopic saline wet mount, and a potassium hydroxide (KOH) whiff test. Three types of vaginitis were assessed: *candida*, bacterial vaginosis, and mixed infections (VVC and BV).

CHAPTER 3

METHODS

Research Design

This was an experimental, double-blind study. One group of women, the control group, was given an oral placebo to take on a daily basis. The other group, the study group, was given a zinc supplement to be taken daily. Comparisons were made between the two groups to determine if zinc supplementation reduced the incidence of recurrent vaginitis. The independent variable in the study was zinc supplementation. The dependent variable was the incidence of vaginitis. The research assistant used a coin toss method to determine which group the first participant would enter. Thereafter participants were placed in alternate groups.

Sample and Setting

Sample

Women were identified through visits over the last 12 months to the researcher's private practice. They were asked to voluntarily participate in the study. Selection criteria limited participation to non-pregnant, sexually active females, between the ages of 18 - 45. Participants had no history of diabetes, were not pregnant, were not on any daily medications other than contraceptives, anti-depressants, non-steroidal anti-inflammatory drugs, antacids, or anti-hypertensives (in people with normal kidney function), were pre-menopausal, and were not intrauterine device (IUD) users. IUD use is thought to affect normal vaginal flora (Star, 1995). Criteria for exclusion included conditions known to influence zinc status such as inflammatory diseases, recent myocardial infarction, recent surgery, chronic debilitation or malabsorption states, chronic infections other than vaginitis,

and corticosteroid use (Edman et al., 1986). Chronic health conditions may affect absorption and retention of zinc in the body. Therefore, women with chronic health conditions were excluded from the study.

Participants were consumers who had been seen in the researcher's practice in the past year and found to have had at least two episodes of clinician diagnosed bacterial vaginosis or yeast infection, or mixed vaginitis in a previous 6 month time frame.

Setting

The study site was a private practice obstetrics and gynecology office in a rural community in a Midwestern state. This was a free standing, one floor, handicap accessible building. The facility includes six modern exam rooms, and a lab where microscopies were performed.

Instruments

Instruments of measurement in this study were the wet mount, the potassium hydroxide (KOH) whiff test, the pH nitrazine paper test to diagnose vaginitis, and the 24-hour diet recall to estimate average daily zinc intake. The researcher also occasionally used a laboratory culture if it was deemed necessary. "If a wet mount has a questionable result, a laboratory culture can help confirm or deny the presence of vaginal pathogens" (Sobel et al., 1998). Anyone unresponsive to treatment received a urogenital culture. "Fungal culture results will corroborate microscopic findings and are more sensitive than microscopic examination to detect yeast" (Nyirjesy, 1997, p. 19).

24-Hour Diet Recall

To estimate the subject's dietary zinc intake, the standard 24-hour diet recall method was used. The study participants were asked to identify how many servings of all foods they consumed in the last 24 hours, providing this was a normal intake for them. None of the participants reported that the previous 24 hour time period had been atypical. This recall determined if the subject's intake fell within the average range of zinc consumption (8.1 mg daily). Dietary recall was documented on the subject's second visit, and was administered

by the researcher. Participants were asked to estimate the size of their servings and given visual aids of a 2 cup measure, a 1 cup measure, and a one-half cup measure. Their average zinc consumption was then estimated using information obtained from Bowes & Church's food values of portions commonly used (Pennington, 1994).

Diagnosis of Vaginitis

Patient history. The patient history for diagnosing vaginal infections included a description of vaginal symptoms with duration, sexual activity, current medical conditions and medications (including use of herbs, vitamins, and over the counter preparations), contraceptive methods (including use of condoms, spermicides or treated condoms), history of STDs or vaginitis, hygiene habits, recent change in detergents or soaps, recent episodes of douching, a description of aggravating or alleviating factors, family history of diabetes, social history, and a menstrual history (Plourd, 1997; Star, 1995).

Diagnosis of BV included an accurate and thorough patient history, bimanual pelvic examination, pH test, wet mount, and KOH whiff test (Plourd, 1997; Association of Professors in Gynecology and Obstetrics [APGO], 1996). Diagnosis of BV is made by the presence of 3 of the following 4 objective findings: abnormal discharge, elevated pH, amine odor, and the presence of clue cells by microscopy (Star, 1995). The wet mount/KOH slide of a patient with BV will have an obvious lack of lactobacilli, clue cells, WBCs, and may have high concentrations of anaerobic bacteria such as *Bacteroides, Mobiluncus*, and *Gardnerella vaginalis*. How this imbalance of lactobacilli and anaerobic bacteria causes BV is not yet known (Selleck, 1997). Although unreliable alone for diagnosis, subjective symptoms of BV include a mild to moderate discharge with a fishy odor (particularly worse after intercourse), and mild vulvar irritation or burning.

Diagnosis of VVC included an accurate and thorough patient history, pelvic exam, pH test, and wet mount with KOH. A vaginal culture is also helpful when patients report symptoms of VVC, but have a negative wet mount. VVC does not raise vaginal pH. Subjective symptoms of VVC include vulvar itching and an increased white clumpy or

flocculent discharge. The patient with VVC may also complain of dyspareunia, burning with urination, and soreness (Sobel, Faro, Force, Foxman, Ledger, Nyirjesy, Reed, & Summers, 1998).

Physical exam. Physical examination to diagnose vaginal infections included a close inspection of the vulva, vaginal walls and cervix for the presence of lesions, discharge or erythema. The specula was lubricated with a small amount of warm water only as KY jelly can affect pH readings. The vaginal discharge was examined for color, consistency, odor, whether it was coming from the cervix, and if it was adherent to the vaginal walls. BV discharge is usually thin, homogenous, white, gray, adherent, malodorous, flocculent, and sometimes increased (APGO, 1996). Signs of BV include: elevated vaginal pH, mild to moderate milky discharge, with a fishy or amine odor.

Objective signs of VVC are vulvar edema and erythema. The vulva and vagina are often red, inflamed, and excoriated due to scratching. The uterus, adnexal, and rectovaginal exam are within normal limits (Star, 1995). A vaginal discharge is common, and tends to be thick, clumpy and white (Youngkin & Davis, 1994). Wet mount will show normal flora, blastospores, and 40% - 50% of patients will be positive for pseudohyphae on the saline side. On the 10% KOH side, the positive pseudohyphae will increase to 60% - 90% of patients. See discussion immediately following for description of preparing a wet mount/KOH prep. There will also be a decreased lactobacilli population (Sobel, 1997). WBCs are present in mixed infections, as well as both yeast hyphae and clue cells.

Wet mount/KOH prep. A wet mount involves examining vaginal discharge under the microscope. The wet mount is considered to be the key diagnostic test for BV (Selleck, 1997). Preparation of a wet mount involves preparing two slides: one with 2 drops of normal saline, the other with 2 drops of a 10% - 20% solution of KOH (potassium hydroxide). Two separate swabs of vaginal secretions from the vaginal walls are obtained using a clean wooden spatula. Use of Dacron or cotton swabs was avoided because the

fibers may sometimes be mistaken for yeast hyphae on microscopic examination (Selleck, 1997).

The first sample of vaginal secretions was applied on the saline slide using two or three rotations of the spatula to ensure a dilute sample. The second sample of vaginal secretions was applied to the KOH slide. Ten to fifteen rotations of the spatula in the KOH was used to assure a fairly concentrated sample (APGO, 1996). A "whiff test" with the KOH slide was performed immediately. The KOH-vaginal fluid mix was sniffed to assess for a strong, amine odor. The presence of this odor occurs in abut 50% - 75% of all patients with BV (APGO, 1996).

The slides were then examined within 5 minutes of preparation under both low power (4x - 10x objective) and high power (≥ 40x objective) settings. At low power, the saline slide was observed for the presence of white blood cells (indicative of Trichomonas), mobile pear-shaped organisms with a tail (Trichomonas), yeast buds or pseudohyphae, and lactobacilli (long, rod-shaped). The KOH sample was examine for clue cells and pseudohyphae. A clue cell is a vaginal epithelial cell with clumps of bacteria adherent to the edges. At least 20% of observed epithelial cells should be clue cells to be of diagnostic significance for BV (Sobel, 1997). "A normal vaginal pH, and a negative whiff test eliminate 95% of all cases of BV" (Sobel, Personal Communication, 1998). A normal vaginal slide would contain only vaginal epithelial cells and lactobacilli.

Wet mount interpretation depends on the skill of the clinician. The researcher has been preparing and interpreting wet mounts for several years. She has also had two training sessions at Wayne State University in 1998 with Dr. Jack Sobel, an internationally known expert in the field of vaginitis. The researcher was the only clinician interpreting the wet mounts, thus consistency was maintained.

Nitrazine paper pH test. The pH test provides a quick indication of what type of infection the patient may have. A pH greater than 4.5 can indicate BV. *Candida* (yeast) infections usually result in a pH of less than 4.5 (Sobel, 1997). Douching, water, recent

intercourse, lubricants, cervical mucous, semen, spermicides, condoms, menstrual secretions, and amniotic fluid can all result in altered pH (Sobel). The subjects were asked to refrain from intercourse for 24 hours before their exams. They were questioned about recent intercourse prior to their exams and all subjects denied this. To obtain a pH specimen, the posterior and lateral fornices of the vagina were swabbed and the specimen was placed on the pH paper. The cervical os was avoided due to cervical mucous lowering the pH value. Evaluation of the pH level was done by comparing the color of the pH test strip to the color key on the test strip container (APGO, 1996).

Procedure

The researcher kept a record for the past 12 months of women who presented to her with recurrent vaginitis (2 or more infections in a six month time frame). All women presenting with complaints of a vaginal infection underwent a physical exam, patient history, KOH whiff test, wet mount, and pH test. Based on the researcher's diagnosis, they were treated with an appropriate medication (see Appendix F for office protocol for treating vaginitis).

The women in the practice who had been identified as having recurrent vaginitis were contacted by telephone by the researcher or the research assistant. The women were invited to participate in the study. A letter that explained the procedure in more detail was sent to the women who stated they were interested in participating in the study. The women were scheduled a 30 minute appointment with the researcher to review the consent form in detail, answer any questions, receive their initial exam (which included a patient history, physical exam, wet mount, pH test, and KOH whiff test), and receive their supplements or placebos.

After selection and informed consent were obtained, subjects were randomly placed in either the study group or the control group by the research assistant using a coin toss method initially, and then placed in alternating groups.

The zinc capsules were removed from their original packaging by the research assistant and placed in plain bottles obtained from a pharmacy labeled "B". The placebo capsules were prepared by a local pharmacy and placed in plain bottles labeled "A". The placebos were gelatin capsules filled with corn starch. The research assistant had exclusive knowledge as to which bottles contained the zinc, and which bottles contained the placebos. The researcher did not have access to any information that revealed which capsule was the zinc and which was the placebo.

Each participant was designated an individual sheet in the researcher's notebook to carefully track all data (Appendix B). This data included: age, race, name, patient identification number, whether they were taking capsule A or capsule B, diet recall and average zinc intake, any medications, vitamins, or herbal supplements they may have been on during the course of the study, number and type of vaginal infections, treatment used, 3 and 6 month count of capsules, results of initial, 3, and 6 month wet mounts, KOH whiff tests, pH tests.

Participants were asked to present to the researcher, free of charge, once every 3 months for a wet mount, pH test, KOH whiff test, and physical exam. Appointments were scheduled that were convenient for the participants. The participants were also asked to present at any time they had symptoms of a vaginal infection. These instructions were given to them in writing, and reviewed with them verbally by either the researcher or the research assistant. Findings of the 3 wet mounts, pH tests, and KOH whiff tests allowed the researcher to detect any vaginal infections that may have gone unnoticed by the subject. A wet mount, pH test, KOH whiff test, and physical exam at the time of vaginal symptoms established the diagnosis of a vaginal infection.

When subjects returned for their 3 month interval visits, they were asked to bring in their bottles of capsules to help determine if they were taking the capsules as directed. The research assistant manually emptied and counted the remaining capsules and documented this in her notebook. The researcher did not observe this activity. If it appeared the

participant was not taking the capsules regularly, she was counseled by the research assistant. At 3 months an interim history was obtained by the researcher, as well as a diet recall. Questions asked were directed at any health changes, change in sex partners, major life stressors (job change, moves, divorce), or dietary changes. These data were entered in the researcher's notebook.

The final 6 month visit included a physical exam, wet mount, pH test, KOH whiff test, and another interim history. Any change in the subject's situation was again noted in the researcher's notebook. If physical exam or patient history revealed signs or symptoms of a STD, appropriate follow up would have been obtained. The researcher found no subjects at risk for STD's by history or physical exam. No changes were implemented in the procedural process during the study.

In order to minimize effects on absorption from other nutrients or supplements, participants were instructed to take either the zinc 30 mg capsule or placebo at bedtime with a food from an approved food list (see Appendix C). Foods containing calcium or iron have the greatest effect on altering zinc absorption. Therefore, women were asked to take their vitamin, mineral, or herbal supplements in the morning. At bedtime, or at least two hours after meal consumption, they were instructed to take their zinc supplement or placebo with either a glass of water alone, or with foods that do not contain significant amounts of calcium or iron.

Vaginal infections can be a secondary effect of taking antibiotics. All study participants were, therefore, asked to report any antibiotic therapy they used during the course of the study. This was recorded in the researcher's notebook. If a subject was found to have a vaginal infection during the study, she was offered a standard treatment (see Appendix F) and remained in the study. Both study groups were treated the same.

Human Subjects Protection

This study required that half the participants take 30 mg of zinc orally per day. There are no known risks involved in using zinc supplementation at this level (Walsh et al.,

1994). The dosage of 30 mg was chosen because it is well tolerated, there is no danger of toxicity, and it is readily available. This is well below the 300 mg dose where immuno-suppression is suspected (Walsh et al.). Women were told that if they were in the placebo group, they were taking a gelatin capsule filled with corn starch.

Subjects were assured of confidentiality and anonymity when they signed their consent form (see Appendix A). They were assured that participating in the study would not interfere with health care provided through the researcher's office. They were advised of any risks (zinc can cause nausea in some subjects). The list of participant's names was later destroyed for confidentiality.

CHAPTER 4

RESULTS/DATA ANALYSIS

Data Analysis

Maintaining a healthy vaginal microecology is an important factor in preventing vaginitis. Because zinc deficiencies can lead to impaired immune functioning, correcting a presumed zinc deficiency may help maintain a normal colonization of lactobacilli in the vagina, thereby reducing the incidence of recurrent vaginitis. The purpose of this experimental double-blind placebo study was to evaluate the effect of oral zinc supplementation on the incidence of vaginitis (either VVC or BV) in women ages 18 - 45 that are prone to recurrent vaginitis. The statistical software program used was Minitab®. The level of significance was set at $p = .05$.

Characteristics of Subjects

Fifty-six women were initially identified as meeting the inclusion criteria for the study. These women were contacted by phone and asked to voluntarily participate. Forty-one women agreed to participate in the study. All women were Caucasian and between the ages of 18 - 45. All of them lived within a 40 mile radius of the researcher's office. At each visit subjects were asked about specific stressors in their life, i.e.; job change, divorce, separation, or death in the family. None of the participants stated they experienced any of these stressors during the study.

Medications used by participants during the study included anti-depressants, oral contraceptives, Depo-Provera contraception, pain relievers, antacids, and antibiotics. Herb use among the participants was infrequent, however some participants reported using St. John's Wort, fish oil, cranberry extract, echinacea, and acidophilus. Only one participant in

the placebo group reported use of acidophilus. None of the participants had experienced a recent birth or termination of pregnancy. None reported a change in sex partner or any significant changes in their diet, health status, or weight. The participant's occupations were varied (e.g. accountants, waitresses, homemakers, teachers, bartenders, and clerical workers).

Participants who withdrew. Fifteen participants withdrew from the study after the initial visit, leaving a total of 26 participants. The women who withdrew were of similar age to the remaining subjects: m = 28.88 years in the placebo group (n = 8), and m = 29.83 years in the zinc group (n = 7). The number of previous vaginal infections was similar as was as their vitamin and contraceptive use. Table 1 compares several characteristics of participants who stayed in the study with those who withdrew.

Table 1

Comparison of Participants with Those who Withdrew (N = 41)

	Study Participants N = 26	Subjects who Withdrew N = 15
Age range	18 - 44 years	17 - 39 years
Contraceptive users	12 (m = 2.2)	5 (m = 3)
Vitamin/Calcium users	10 (m = 2.6)	5 (m = 3)
Prior vaginal infections	m = 2.9	m = 2.6

Potential Intervening Variables

This section will examine the characteristics of the 26 women who remained in the study. In order to determine if there were any potential intervening variables that were different between the placebo group and the zinc group, nine characteristics of the participants were examined. Table 2 summarizes these characteristics. It appeared there were no differences in the participant's age, zinc intake, prior vaginal infection rate,

antibiotic use, or vitamin use. However, three potential intervening variables were identified: compliance (number of pills missed), contraceptive use, and extra visits. One participant in the zinc group was found to have 9 infections and 7 extra visits, and therefore considered to be a potential outlier. A statistical analysis done without that subject did not change the results.

Table 2

Characteristics of Study Participants (N = 26)

	Placebo Group n = 11	Zinc Group n = 15
Age	31.6 years[a]	29.7 years[a]
Daily dietary zinc intake	6.1 mg[a]	5.68 mg[a]
Pills missed during study	18.2[a]	8.3[a]
Vaginal infections in 6 months prior to study	2.9[a]	2.9[a]
Extra visits for vaginal complaints	3	15
Persistent infections	1	3
Courses of antibiotics during study	4	7
Vitamin and/or calcium users	4	6
Oral contraceptive or depo-provera users	4	8

[a] Expressed as a mean.

In order to determine if the three potential intervening variables were statistically significantly different between groups, a 2 sample t-test analysis was performed on each variable. None were found to be statistically significant. Table 3 shows the statistical data of these three variables.

If a woman was found to have a persistent vaginal infection during the study, a urogenital culture was obtained. This occurred three times in the zinc group. One culture was positive for E. coli and she was treated with a seven day course of cephaloxin; the other

two confirmed a heavy growth of gardnerella and *Candida* albicans. A persistent vaginitis was found once in the placebo group; that subject had one urogenital culture confirming *Candida* albicans in a mixed infection, and another culture confirming gardnerella in a diagnosis of BV.

Table 3

Potential Intervening Variables

	Mean	SD	t	df	p
Compliance					
Placebo	18.2	15.6			
			1.71	19	0.10
Zinc	8.3	13.1			
Contraceptive Use					
Placebo	0.364	0.505			
			1.53	21	0.14
Zinc	0.667	0.488			
Extra Visits					
Placebo	0.273	0.647			
			1.41	18	0.18
Zinc	1.00	1.85			

Hypothesis Testing

A summary of the incidence of vaginal infections prior to and during the study can be found in Table 4. In order to compare the incidence of vaginitis after the treatment (zinc or placebo) effect, the number of prior vaginal infections was combined with the initial visit data. Since no treatment had been administered at the initial visit, if participants were diagnosed with a vaginal infection at that time, that infection was included with the prior infections.

Statistical Analysis

In order to test for differences in the incidence of vaginal infections between the placebo group and the zinc group at post-treatment, a t-test analysis for independent means

was performed. Table 5 shows this information. There was no significant difference found ($p = 0.16$). The research hypothesis that women between the ages of 18 - 45 prone to vaginal infections who take 30 mg of zinc gluconate daily would have fewer cases of vaginitis than women who do not take a supplement during a six month time frame was rejected.

Table 4

Summary of Incidence of Vaginal Infections in Participants (N = 26)

Group	BV		Yeast		Mixed		Total	
Zinc (n=15)	Prior	Study	Prior	Study	Prior	Study	Prior	Study
1 Infection	2	4	1	3	3	1	6	8
2-3 Infections	8	3	3	1	2	2	13	7
< 3 Infections	2	1	2	0	0	0	4	1
Total Infections	31	14	19	5	7	6	57	25
Placebo (n=11)	Prior	Study	Prior	Study	Prior	Study	Prior	Study
1 Infection	1	1	2	1	1	1	4	3
2-3 Infections	7	1	2	2	0	0	9	3
< 3 Infections	1	0	1	0	1	0	3	0
Total Infections	23	3	10	5	5	1	38	9

Table 5

Statistical Analysis of Placebo Group and Zinc Group (N = 26)

Vaginitis Episodes	Total	Range	Mean	SD	df	t-value
Placebo (n=11)	9	(0 - 3)	0.82	1.06	22	1.45
Zinc (n=15)	25	(0 - 7)	1.67	1.88		

On further examination of data from the zinc group, it appeared there was a difference in the mean incidence of yeast, dropping from 19 prior to the study ($m = 1.26$) to 5 following zinc supplementation ($m = 0.33$). A paired one sample t-test analysis

confirmed a significant decrease in the incidence of VVC in women taking 30 mg of zinc gluconate daily ($t = 2.23$, $p = 0.021$).

CHAPTER 5

DISCUSSION AND IMPLICATIONS

Discussion

Results

The research hypothesis for this study was that women between the ages of 18 - 45 prone to vaginal infections who take 30 mg of zinc gluconate daily would have fewer cases of vaginitis than women who did not take a supplement during a six month time frame. It was found that the overall occurrence of vaginitis between the placebo group and the zinc group at post-treatment was not significantly different ($p = 0.16$). Thus the hypothesis was rejected. However, a significant decrease in the incidence of VVC infections was noted in the zinc group ($p = 0.021$).

Per a 24 hour dietary recall, the 26 women participating in the study had a mean daily zinc intake of 5.86 mg, while the RDA for zinc is 12 mg per day. This group of women with documented recurrent vaginal infections can be classified as having inadequate zinc intake based on the 24-hour diet recall. It is interesting to note that the participant with the most documented vaginal infections (14 prior to and during study) had the lowest zinc intake (0.13 mg/day). This subject was undernourished based on her 24-hour diet recall. She reported consuming only one small meal that day and on typical days.

Neuman's Model

Even though the research hypothesis was rejected, some data from the study seem to support the theory that zinc has impact on the vaginal ecosystem. Women in this study had inadequate zinc intakes and had recurrent vaginal infections. Women treated with zinc had a decrease in the incidence of VVC. Recalling the vaginitis theory based on Neuman's

Model (see Figure 2), the internal lines of resistance represent the vaginal ecosystem. Part of this ecosystem is T cells, which are responsible for attacking foreign organisms. Walsh, et al., (1994) stated that "Even a mild or marginal zinc deficiency in humans is characterized by decreased natural killer cell activity and alterations in T cell subpopulations". Therefore, the lines of resistance may be weakened by inadequate zinc intake.

The flexible line of defense acts as a buffer to prevent invasion of stressors, in this case vaginitis, and can be affected by nutrition. A woman with good overall nutrition is likely to have stronger defenses against illness than a woman with inadequate or poor nutrition. Using zinc supplementation may help strengthen the flexible line of defense. There may be other factors not yet identified that can strengthen a woman's defenses against recurrent vaginal infections.

Inferences from Literature Review

The researcher found no significant difference in the overall vaginal infection rate between the placebo group and the zinc group at post-test. There was however a decrease in the rate of yeast infections in the zinc group from 19 to 5. One could theorize that the lactobacilli in the vagina have more impact on yeast infections than BV. In fact, Edman et al. (1986) found that zinc deficiency reduced the immunity of mice to *Candida* albicans, while a zinc excess was a protective factor.

There have been no studies done using zinc as a prevention for vaginitis. However, if the review of literature is re-examined, several consistencies can be found. Scheer (1998) stated that a zinc deficiency could lead to impaired immune function. Edman et al. (1986) found that zinc deficiency depresses a number of cellular immune functions, including T cell proliferation. Walsh et al. (1994) state that depression of B and T cell formation is manifested as a progressive increase in opportunistic infections, especially *Candida* albicans. Nyirjesy (1997) states that women with recurrent VVC have been found to have reduced antigen specific T-lymphocyte reactivity. All of this literature supports the theory

that zinc deficiency reduces the T cell population in the vaginal ecosystem, thereby placing a woman at risk for infection with *Candida* albicans.

There are three documented cases of zinc being used as a treatment for recurrent or resistant trichomonas. Andrews et al. (1994) used bacitracin plus zinc, Houang et al. (1997) used zinc sulfate douches plus metronidiazole, and Willmott et al. (1983) used oral zinc sulfate. All three of these cases were successful at eradicating the trichomonas with the addition of zinc when conventional therapies had failed.

There have been prior studies on using zinc as a topical treatment for resistant vaginal infections and there have been prior studies on zinc deficiency and recurrent vaginitis. However, this is an original study on supplementing zinc in women prone to recurrent vaginal infections, so the researcher had no other study with which to compare these results.

Threats to Internal Validity

The procedure was assured validity through the use of the double-blind technique. Neither the researcher nor the study participant knew if the subject were taking a zinc supplement, or a placebo. Asking the participant to bring in her capsule bottle at the appropriate times to determine if she took them correctly protected internal validity. If a variability existed in the daily consumption of capsules, close record of this was kept in the researcher's notebook and the participant was counseled.

Threats to internal validity of the study were of most concern in the area of attrition. It was assumed that most women who had problems with recurrent vaginitis would be motivated to find solutions to their problems and this motivation would help with retention and follow through. Women are often frustrated that the health care system has little to offer for prevention of recurrent vaginal infections and therefore may have been willing to try alternative methods of treatment or prevention. Most of the women contacted initially agreed to participate in the study, yet the attrition rate was high.

It was hoped that the sample size of at least 30 participants in both the zinc and placebo groups would provide a margin for attrition with enough remaining numbers to perform meaningful statistical analyses. However, with the small sample size and the high attrition rate, that did not happen. Randomizing whether the participant received the zinc or placebo helped assure validity. Selection criteria facilitated similar subjects, and randomization of groups again minimized these threats. Subjects were selected based on no other risk factors being present.

The attrition rate for the study was 37%, or 15 of 41. Because participants were not required to inform the researcher nor asked why they decided not to participate, specific reasons cannot be quoted in all cases. Three of the participants became pregnant. Several who dropped cited reasons such as not remembering to take their capsules, being unable to follow the guidelines for taking capsules, or not having enough time to schedule the required visits. One participant was dropped because it was discovered that she was 17 years old and below the age of informed consent.

The Hawthorne effect was minimized because both groups of women being studied received an intervention. They both took pills, and both groups came in at three month intervals to be examined.

History effects on the participants were minimized because they were not asked to change any of their current dietary or behavioral habits, and were not encouraged to make lifestyle changes such as changes in sexual practices. Becoming pregnant was a maturation threat that could not be controlled. If a participant became pregnant during the study, she was withdrawn. The researcher stayed in contact with the study participants throughout the six months. The researcher or research assistant collected all pertinent data to describe what happened during the course of the study (see Appendix B). Participants were offered free office calls for symptoms of vaginitis while they were enrolled in the study.

Limitations

This study was limited by several factors. Small sample size was a major limitation (N=26). This factor placed severe limits on performing meaningful statistical analysis. Absence of a plasma or urinary zinc level measurement in the subjects to document zinc deficiency was a concern. This factor was cost-prohibitive in this study environment. Providing zinc supplementation at only one dosage also limited the range of this study as different effects may be noted at different dosages.

The study of nutrient deficiencies is a complex phenonemon. Use of the 24 hour diet recall gives the researcher only a small sample of a subject's true dietary intake. There are multiple relationships between nutrients that can affect absorption and utilization. The only such factor this study attempted to control was the relationship between calcium and zinc. Factors that were not controlled for because of the small sample size were the use of herbs, contraceptives, vitamins, antacids, and sexual practices.

An assumption made in this study was that participants were heterosexual and monogamous. No control could be made for participant's sexual partners changing sexual partners. Another assumption was that by supplementing zinc at 30 mg (which is over the RDA of 12 mg), subjects would be adequately nourished. An inadequate zinc intake may represent only one nutrient deficiency in a subject's diet. How other nutrient deficiencies may affect vaginitis has not been researched. A larger study would enable a researcher to be more selective or controlling in regards to some of these factors.

During the data collection process, the researcher did ask about recent intercourse before the physical exam as semen can alter pH readings. However, the participants were not questioned about recent use of douches, or sexual practices. Douching can alter pH readings. Oral/genital sex can increase the occurrence of BV. The participants were discouraged from using douches as part of the general hygiene information given to patients with recurrent vaginitis. However, objective measures for vaginitis may have been affected by the lack of documentation in regards to douching and sexual practices.

Suggestions for Further Research

A future study with a larger sample size would be of great benefit to nursing research. Based on the researcher's experience with this study, several modifications can be suggested. The next researcher may want to limit the study to women with just recurrent VVC as this study showed a decrease in VVC in women supplementing zinc. Or she may want to expand to include greater numbers of subjects with recurrent or resistant trichomoniasis as prior research has shown zinc to be effective at eradicating this infection. Having several study groups, taking different dosages of zinc such as 15 mg, 30 mg, 45 mg, 75 mg up to 100 mg, would also provide needed information. This study could also incorporate serum zinc measurements to supply additional data related to zinc deficiency. It is possible that the effects of zinc supplementation are not evident in a six month time frame. A study for a one year period of time may provide different results.

It is also possible that starting with a population prone to vaginal infections skewed the results. A study encompassing a random sample of all women might decrease the incidence in general and provide better insights into the effects of zinc supplementation.

A recent study shows a possible link between cigarette smoking and BV (Brunk, 1999). Therefore, further research should include data collection on the incidence of tobacco use. Using a topical zinc treatment for recurrent vaginitis, such as a suppository or cream, may also be worth investigating.

Implications

Application to Practice

It is estimated that one-half of adult women in the US consume less than 7.5 mg of dietary zinc daily (Wood & Zheng, 1997). The US RDA for zinc in adult women is 12 mg. The study participants consumed an average of 5.86 mg daily, and were known to suffer from recurrent vaginal infections. Since a decrease in the incidence of VVC was noted, and no long-term detriments to zinc supplementation are documented, women might benefit from daily zinc supplement to prevent recurrent VVC. The noted zinc deficiency alone in

the subjects, known to be prone to recurrent vaginal infections, suggests the need to increase zinc either by improved nutritional intake or by supplementation.

Summary

Although this study did not show a statistically significant difference in the incidence of overall vaginitis in women taking a zinc supplement versus a placebo, it is still intriguing to note that women prone to vaginal infections were found to have inadequate zinc intake based on a 24 hour diet recall, taking in on average less than half the RDA of zinc. It is also noteworthy that women supplementing zinc did experience a decrease in the incidence of recurrent VVC. Clinically, if one encounters a woman with recurrent vaginal infections, it may be prudent to administer a 24-hour diet recall to investigate the possibility of a zinc deficiency.

Vaginitis is a condition of multiple and interrelated causation. One potentially contributing factor is inadequate zinc intake. While this factor was not statistically supported in this study, this researcher recommends a larger, more complex and longitudinal study.

A main function of the nursing profession is to assist the client to recognize relationships among the physiological, psychological, sociocultural, developmental, and spiritual components of life. In regards to recurrent vaginitis, the advanced practice nurse needs to go beyond examination of the woman's vaginal symptoms, and explore with the client her lifestyle practices. Hygiene, sexual practices, smoking, and poor dietary habits may all be contributing factors to recurrent vaginal infections.

APPENDICES

Appendix A

Subject's Consent Form

I understand that this is a study of recurrent vaginal infections. It is estimated that one-half of women in the United States may not get enough zinc in their diet. Zinc deficiency is associated with the body's immune system not working well. The knowledge gained through this study is expected to help nurses and physicians provide helpful information to women in preventing recurrent vaginal infections.

I also understand that:

1.) Participation in this research study is voluntary.

2.) Participation in this research study will involve me taking either a zinc supplement (30 mg of zinc) as a capsule, or a placebo capsule (a gelatin capsule filled with corn starch) on a daily basis.

3.) Calcium and iron can decrease the amount of zinc absorbed by your body. Therefore, your zinc supplement or placebo should be taken two hours before or after any dairy products, calcium supplements, antacids, iron supplements, or multi-vitamins containing calcium or iron. Attached is a list of low calcium and low iron foods which you may take the zinc supplement with.

4.) I have been selected to participate in this study because I have had at least two clinician diagnosed vaginal infections in a six month time period, or one infection that has not gotten better.

5.) There are no known physical or emotional risks involved with taking zinc supplements.

6.) I will need to be examined by the researcher three times in the six month study. Once to receive instructions and the supplement, once at three months into the study, and again at six months. These visits will be free of charge.

7.) There will be no cost, other than transportation to and from the office, involved in my participation.

8.) The information I provide, and the results of my vaginal exam will be kept strictly confidential. Data will be coded so that identification of individual participants will not be possible.

9.) A summary of the study results will be made available to me upon request. Please initial here if you would like to receive a copy of the study results _____.

10.) If I develop symptoms of a vaginal infection, I am free to visit the researcher, or another clinician, to seek treatment. These visits for vaginal symptoms will be free of charge during the six month study. The

researcher will need to have any such infection recorded for her study, so I will need to provide that information to her if care was sought through another health care provider, or if I used over the counter remedies.

11.) I will also need to report to the researcher if I take any oral antibiotics during the course of the study.

I acknowledge that:

1.) I have been given the chance to ask questions regarding this research study, and these questions have been answered to my satisfaction.

2.) In giving this consent, I understand that my participation is voluntary and I may stop at any time using the postcard with my patient identification number provided by Bonnie Bartz without it affecting the care I receive from her, the physicians, or staff at Alpena OB/GYN.

3.) I give permission to the researcher to release information obtained in this study to scientific literature. I understand that I will not be identified by name.

4.) Phone numbers of the researcher and the chairperson at Grand Valley State University (GVSU) Human Research Review Committee are listed below. I understand I may contact them for questions about the research. Researcher: Bonnie Bartz (517) 356-0504. Chairperson: Dr. Phyllis Gendler (616) 895-3516. For questions about your rights in the study please contact Paul Huizenga at (616) 895-6611.

5.) I acknowledge that I have read and understand the above information, and that I agree to participate in this study.

_____ _____

Participant's Signature Witness

_____ _____

Date Date

Appendix B

Researcher's Notebook

Pt. ID.#_____

Name _____

Address _____

Phone # _____

DOB _____ Age _____

Race _____ G _____ P _____ AB_____

Occupation _____

Medical Problems _____

Food Allergies_____

Contraceptive Use_____

Current medications, herbs, vitamins, OTC products_____

_____

Documentation of previous episodes of vaginitis

Date _____	Date _____
Symptoms_____	Symptoms_____
_____	_____
pH_____	pH_____
KOH whiff _____	KOH whiff _____
Wet Mount _____	Wet Mount _____
Physical exam _____	Physical exam _____
_____	_____
Treatment _____	Treatment _____
Taken as directed?_____	Taken as directed?_____

Pt. ID.#_____

Date _____	Date _____
Symptoms_____	Symptoms_____
_____	_____
pH_____	pH_____
KOH whiff _____	KOH whiff _____
Wet Mount _____	Wet Mount _____
Physical exam _____	Physical exam _____
_____	_____
Treatment _____	Treatment _____
Taken as directed?_____	Taken as directed?_____

Study Consent Form Signed _____(Date)

Instructions Reviewed with Participant_____(Initials)

Capsule A or B

Exam Data Pt. ID. # _____

Initial Exam _____(Date)

Symptoms_____

pH _____ KOH Whiff Test _____

Wet Mount _____

Physical Exam _____

Treatment _____

Taken as directed? _____

Intercourse or contraceptive use within 24 hours prior to exam? _____

Three Month Exam _____(Date)

Symptoms_____

pH _____ KOH Whiff Test _____

Wet Mount _____

Physical Exam _____

Treatment _____

Taken as directed? _____

Intercourse or contraceptive use within 24 hours prior to exam? _____

Final Six Month Exam _____(Date)

Symptoms_____

pH _____ KOH Whiff Test _____

Wet Mount _____

Physical Exam _____

Treatment _____

Taken as directed? _____

Intercourse or contraceptive use within 24 hours prior to exam? _____

Pt. ID.#_____

Significant Events During Study

Episodes of vaginal symptoms

_____(Date)

Symptoms_____

pH _____ KOH Whiff Test _____

Wet Mount _____

Physical Exam _____

Treatment _____

Taken as directed? _____

_____(Date)

Symptoms_____

pH _____ KOH Whiff Test _____

Wet Mount _____

Physical Exam _____

Treatment _____

Taken as directed? _____

_____(Date)

Symptoms_____

pH _____ KOH Whiff Test _____

Wet Mount _____

Physical Exam _____

Treatment _____

Taken as directed?_____

Pt. ID.#_____

Medications Taken

Date	Date
Diagnosis	Diagnosis
Prescription	Prescription
Length of treatment	Length of treatment

Date	Date
Diagnosis	Diagnosis
Prescription	Prescription
Length of treatment	Length of treatment

Major Diet Changes

Date _____

Description _____

_____

Change in Sex Partner

Date _____

Date _____

Major Change in Life Stressors

Date _____ Incident _____

Date _____ Incident _____

Date _____ Incident _____

Appendix C

Patient Instructions

Dear Potential Study Participant,

Thank you for considering being involved in this study. As a sufferer of recurrent vaginal infections, you know first hand the pain and frustration that comes with this illness. One of the most frustrating things for me, as a nurse, is not being able to tell you how to prevent these infections from happening. With this study, I hope to be able to show that zinc supplementation can help break this aggravating cycle. In order for the study to be successful, it is essential that you follow these simple guidelines below. If you have questions or concerns at any time, please feel free to call me or Kendra Robinette at the office (356-0504 or 734-2586).

This study involves two groups of participants. One group will receive a placebo (a capsule with just a filler inside). The other group will receive an actual zinc supplement. There will be three times in the next six months I need to see you. Your first appointment will be to receive your capsules, sign the consent form, answer your questions, and get a vaginal exam done. The second appointment will be at three months into the study. At that appointment, you will need to bring in your capsule bottle. Kendra will be counting your remaining capsules to assure that you are taking them on a regular basis. Please don't forget your bottle. You will receive your second vaginal exam at that time. The third and final visit will be at the completion of the study, in six months. This appointment will be a repeat of the three month appointment. The vaginal exams are being done to determine if your vagina is in a healthy state, or if there is any yeast or bacteria present. I hope to see that over time, the group taking zinc will show an improvement in the overall health of their vaginas.

You will not know if you are taking zinc or the placebo until the end of the study. Neither will I. Kendra is keeping track of that information. That is why your bottles are only marked A or B. It is important in research to keep that data confidential for several

reasons. One is if you knew you were taking a placebo, you would be less inclined to take it on a daily basis. The other is if the researcher knew who was in the zinc group and who was in the placebo group, I might start looking for improvements in the zinc group, and thus skew the data I get from your vaginal exams.

Calcium and iron can make your body absorb less zinc. For that reason, I'm asking you to take your capsule at least two hours before or after you eat any foods, or take any vitamins containing calcium or iron. I suggest that if you take a multivitamin, you take it in the morning. Then, take your zinc or placebo capsule at bedtime either with just a glass of water, or with a food that does not contain calcium or iron. Many antacids contain calcium, so please avoid any antacids for two hours before or after your capsule. Below is a list of low calcium and iron foods you may take your capsule with.

Foods that can be taken with your capsule

Graham crackers	Toast
Soda crackers	Vegetables
Tea (without milk or creamer)	Jell-O
Cookies	Cake
Fruit pie (without ice cream or raisins)	Juice (not calcium enriched)
Prunes	Fruit

If at any time during the study you develop symptoms of a vaginal infection, please call and make an appointment with me. I will see you free of charge for this appointment. If you do have a vaginal infection, you will be given the normal treatment. I will not be able to supply the prescription free of charge. If you have an infection that is not responding to treatment, I may find it necessary to perform a lab culture. I will not be able to supply the lab work free of charge. If you develop a infection on the weekend, or for some other reason, cannot make it in to see me, you may use an over-the-counter treatment, or see

another health care provider, but be sure you report to me any treatments you use during the study.

You have been supplied a postage paid postcard. If for any reason, you wish to no longer participate in the study, simply drop the postcard in the mail. It has only your patient identification number on it. You will not be reprimanded, nor in any way treated differently by me or any of the staff at Alpena OB/GYN. No explanation is necessary.

I know this is a big commitment of time and energy on your part. I want to assure you that all information is confidential, data will be recorded with your patient identification number only, not your name. After the data has been processed, the individual records will be destroyed. I want to thank you for your participation. It is my hope that from this small study, a much larger study can be done that could eventually bring hope and relief to thousands of fellow vaginal infection sufferers.

With thanks and appreciation,

Bonnie R. Bartz
Women's Health Nurse Practitioner

Appendix D

Data Collected for Placebo Group

	pH	Wet Mount	KOH Whiff Test	Symp-toms /Exam Data	Culture Results	Treat-ment
Subject 9222 - Initial visit	4.3	+2 yeast	-	Scant d/c, mild irritation	NA	Diflucan
3 mos. visit	5.3	-	-	-	NA	NA
6 mos. visit	4.5	occ. clue cell	-	-	NA	NA
Subject 2550- initial visit	4.5	+2 clue cells	+	Scant d/c	NA	Flagyl
3 mos. visit	4.0	-	-	-	NA	NA
6 mos. visit	4.5	-	-	-	NA	NA
Subject 5783 - Initial visit	5.5	+3 clue cells	+	green d/c with odor	NA	Flagyl for 7 days then Flagyl 500 mg x 1 with intercourse
3 mos. visit	4.5	-	-	c/o odor	NA	IC
6 mos. visit	-	NA	+	c/o odor and green d/c	NA	Phone interview
Subject 4269 - Initial visit	4.2	+1 clue cells	-	-	NA	NA
3 mos. visit	4..0	+2 WBCs, neg. clue cells	-	-	NA	NA
6 mos. visit	4.3	-	-	-	NA	NA
Subject 5495 - Initial visit	4.8	-	-	-	NA	NA
3 mos. visit	4.5	1 yeast, +1 WBCs	-	-	NA	NA
6 mos. visit	4.3	+3 clue cells	-	-	NA	IC

Subject 10642 - Initial visit	5.0	few RBCs	-	mucousy /creamy d/c	NA	NA
3 mos. visit	5.0	-	-	-	NA	NA
6 mos. visit	4.8	Few WBCs	-	copious mucousy d/c	NA	NA
Subject 3248 - Initial visit	4.8	+2 clues	-	scant milky d/c	NA	Cleocin vag. cream
3 mos. visit	4.5	+ 1 yeast	-	white d/c and itching	NA	Diflucan 150 mg x 3, then Diflucan 100 mg every week x 8 weeks
6 mos. visit	4.2	+ 1 yeast	-	mild itching	NA	NA
Subject 8441 - Initial visit	4.0	+1 yeast	-	Red vulva, scant white d/c	NA	Terazol 7 vag. cream
3 mos. visit	4.5	-	-	-	NA	NA
6 mos. visit	4.5	RBCs	-	spotting	NA	NA
5/99	Self-tx'd	yeast				Diflucan
6/99	Self-tx'd	yeast		After abx.		Diflucan
Subject 3206 - Initial visit	4.3	+4 lactobacilli	-	-	NA	NA
3 mos. visit	not recorded	+2 yeast	-	Scant d/c with itching	NA	Diflucan
6 mos. visit	4.3	+1 yeast	-	Slight itch with scant d/c	NA	Diflucan
Subject 10217 - Initial visit	4.5	+1 clue cells	+	Red vulva with slight irritation	NA	Flagyl
3 mos. visit	5.5	+4 clue cells	+		NA	Metro-gel
6 mos. visit	5.3	+4 clue cells	+	Irritation, milky d/c, odor	*Candida* albicans	Flagyl bid for 7 days, weekly x 4, and Diflucan
7/13/99	5.5	+4 clue	+	Burning,	Heavy	Flagyl for

visit		cells		tearing, odor, cx. red, thick yellow d/c	Gardner-ella	subject and her partner
Subject 10800 - Initial visit	4.5	+4 clues	+	Slight greenish yellow d/c	NA	Cleocin vag. cream
3 mos. visit	4.5	-	-	Yellow d/c with burning	NA	NA
6 mos. visit						

Note. The dosages of vaginitis treatments used were standardized as specified on page 57, unless otherwise specified. Abbreviations: d/c = discharge, mod. = moderate, NA = Not Applicable, WBCs = White Blood Cells, RBCs = Red Blood Cells, IC = Insufficient criteria to provide prescription for treatment.

Appendix E

Data Collected for Zinc Group

	pH	Wet Mount	KOH Whiff test	Symptoms	Culture Results	Treatment
Subject 5756 Initial Visit	4.5	+1 clue cells	-	-	NA	IC
3 mos. visit	4.0	-	-	-	NA	NA
6 mos. visit	4.3	+2 clue cells	-	Scant d/c	NA	IC
3/26/99	Self-tx'd	yeast				Mycelex 7
5/29/99	Self-tx'd	yeast				Mycelex 7
Subject 7873 Initial visit	5.2	+1 clue cells	+	Red vulva	NA	Flagyl
3 mos. visit	4.8	+1 clue cells	-	Mod. milky d/c	NA	IC
6 mos. visit	4.8	+1 clue cells	-	Dry vagina	NA	IC
Subject 5846 Initial visit	4.5	-	-	-	NA	NA
3 mos. visit	5.0	RBCs	-	Spotting, slight burning	NA	NA
6 mos. visit	4.8	WBCs	-	Small d/c	NA	NA
5/14/99 visit	4.8	+4 clue cells	+	+4 yellow d/c, burning	NA	Flagyl
6/7/99 visit	Telephone call		+	Same symptoms	NA	Cleocin vag. cream
Subject 7854 Initial visit	5.0	+1 clue cells	+	Slight d/c with odor	NA	Flagyl x 7 days, then 1 Flagyl po with intercourse
3 mos. visit	5.0	+2 clue cells	+	Slight d/c with odor	NA	Metro-gel
6 mos. visit	4.8	+1 clue cells	+	Slight d/c with odor	NA	Flagyl
Subject 8990 Initial visit	5.5	+1 clue cells	+	Burning, odor	NA	Flagyl
3 mos. visit	4.8	+1 clue cells	-	Burning, itching	NA	Metro-gel
6 mos.	4.5	+1 clue	-	-	Shows	Cleocin

visit		cells			heavy gardnerella	vag. cream (on Flagyl for d/c in stool).
Subject 2316 Initial visit	5.0	+4 yeast, +2 clue cells	+	Yellow d/c, red vulva	NA (Note: pt. cannot tolerate Flagyl)	Cleocin vag. cream and a series of 3 Diflucan
3 mos. visit	4.9	+2 clue cells	+	Burning	NA	Metro-gel
6 mos. visit	4.5	+4 clue cells	+	Burning, d/c, irritation, red vulva	*Candida* albicans	Metro-gel and Diflucan
4/20/99 visit	5.0	+4 clue cells, +1 yeast	+	Burning, d/c with odor	*Candida* albicans	Cleocin vag. cream and Diflucan
5/26/99 visit	5.0	+3 clue cells	+	Burning, itching, d/c	NA	Cleocin and Diflucan
Subject 5022 Initial visit	5.0	+4 clue cells	++	Burning, d/c with odor	NA	Metro-gel
3 mos. visit	4.5	+2 clue cells	-	Copious d/c with minor irritation	NA	Metro-gel
6 mos. visit	NA	Not done - on menses	-	No complaints	NA	NA
Subject 5916 Initial visit	5.3	+1 clue cells	+	Milky d/c with burning	NA	Flagyl
3 mos. visit	4.5	-	-	-	NA	Prophylactic Flagyl and Diflucan at end of menses
6 mos. visit	4.8	+1 clues	+	Slight itch and odor	NA	Metro-gel
4/12/99 visit	5.2	+1 clues	+	Slight odor and irritation	NA	Flagyl
4/26/99	5.3	+1 clue	+	Red vulva,	Shows e.	Cleocin

visit		cells, +1 yeast		scant d/c, odor and irritation	coli	vag. cream and Diflucan, then Keflex
5/25/99 visit	5.0	+2 yeast, +1 clue cells	+	Chunky milky d/c with odor, red vulva	NA	Flagyl and Diflucan
7/26/99 visit	4.3	+4 yeast	-	Itching and d/c	NA	Diflucan x 3, then weekly Diflucan x 12 weeks
8/3/99 visit	5.0	+1 clue cells	+	Burning	NA	Flagyl
8/12/99 visit	4.5	-	-	Itchy d/c	NA	IC
8/30/99	5.0	+1 clue cells	+	Burning	NA	Cleocin vag. cream
Subject 1419 Initial visit	5.0	+4 clue cells	+	-	NA	Pt. declined treatment
3 mos. visit	5.3	+4 clue cells	-	Mod. yellow d/c	NA	Cleocin vag. cream
6 mos. visit	5.3	+1 clue cells, +1 yeast	+	Scant creamy d/c	NA	Flagyl
Subject 42 Initial visit	5.0	+1 clue cells, +1 yeast	-	Slight d/c with itch	NA	Diflucan and Flagyl
3 mos. visit	4.8	-	-	Mucousy yellow d/c	NA	NA
6 mos. visit	4.8	RBCs	-	Spotting	NA	NA
3/25/99 visit	Telephone call	NA	NA	Itching	NA	Diflucan
Subject 11131 Initial visit	4.5	+4 clue cells	-	+4 d/c	NA	Flagyl
3 mos. visit	4.0	-	-	White d/c	NA	NA
6 mos. visit	4.3	-	-	Spotting	NA	NA
Subject 5195 Initial visit	4.8	+3 clue cells	-	Normal	NA	IC
3 mos. visit	5.3	+3 clue cells	-	Thick white d/c, itching	NA	Flagyl
6 mos.	4.0	WBCs	-	Creamy	NA	NA

visit				d/c		
7/25/99 visit	Telephone call	NA	NA	Itching, white d/c	NA	Diflucan
Subject 3238 Initial visit	5.5	-	+	Milky d/c at introitus	NA	Flagyl
3 mos. visit	4.5	Mod. lactobacilli	-	-	NA	NA
6 mos. visit	Not completed					
Subject 3256 Initial visit	5.0	+2 clue cells	-	-	NA	IC
3 mos. visit	5.0	+1 clue cells	-	Spotting	NA	NA
6 mos. visit	4.8	WBCs	-	Mod. yellow/ green d/c	NA	NA
Subject 10054 Initial visit	4.6	+1 clue cells	-	Thick white d/c	NA	NA
3 mos. visit	4.5	-	-	Normal	NA	NA
6 mos. visit	Telephone call	NA	NA	Normal	NA	NA

Note. The dosages of vaginitis treatments used were standardized as specified on page 57 unless otherwise specified. Abbreviations: d/c = discharge, mod. = moderate, NA = Not Applicable, WBCs = White Blood Cells, RBCs = Red Blood Cells, IC = Insufficient criteria to provide prescription for treatment.

Appendix F

Vaginitis Treatment Protocol

The protocol within the practice where the researcher is employed states the appropriate medication for treating a yeast infection is considered to be one of the following: fluconazole (Diflucan) 150 mg single oral dose; terconazole (Terazol) 80 mg vaginal suppository, one suppository nightly for three nights; terconazole vaginal cream (Terazol 7), one applicator vaginally nightly for seven nights; miconazole (Monistat) 2% cream, 5 grams vaginally for 7 nights, tioconazole (Vagistat) 6.5% cream, 5 gram single dose. Any of these treatments are deemed adequate according to research done by Sobel, Faro, Force, Foxman, Ledger, Nyirjesy, Reed and Summers (1998), and Roe and Gudi, (1997).

Appropriate treatment for a bacterial vaginal infection includes: metronidazole (Flagyl) 500 mg orally twice a day for 7 days. Alternative treatments include clindamycin 2% vaginal cream (Cleocin), 1 applicator vaginally nightly for 7 nights; clindamycin 300 mg orally twice a day for 7 days (used only with resistant infections); metronidazole vaginal cream (Metro-gel), one applicator vaginally every night for 5 nights (Roe & Gudi, 1997).

If a woman returned after treatment of vaginitis with complaints of a vaginal infection within 6 months of the initial complaint, she again underwent physical exam, wet mount, KOH whiff test, urogenital culture, and pH. It was also determined that she had taken all medications as prescribed initially. This differentiates between recurrent infection as opposed to inadequate treatment. The urogenital culture helps determine if there are organisms present other than yeast or gardnerella such as E. coli.

Appendix G

Summary of Infections Prior to and During Study - Placebo Group (N=11)

Partici-pant	Prior Infections	Initial Exam	3 month Exam	6 month Exam	Extra Visits	Total Infections During Study
9222	5 BV	Yeast	-	-	-	0
2550	2 Yeast	BV	-	-	-	0
5783	2 BV	BV	-	BV	-	1 BV
4269	2 BV	-	-	-	-	0
5495	3 BV	-	-	-	-	0
10642	4 Mixed	-	-	-	-	0
3248	1 BV, 2 Yeast	BV	Yeast	-	-	1 Yeast
8441	3 Yeast	Yeast	-	-	2 Yeast	2 Yeast
3206	2 BV	-	Yeast	Yeast	-	2 Yeast
10217	2 BV, 1 Yeast	BV	BV	Mixed	BV	2 BV, 1 Mixed
10800	1 BV, 1 Mixed	BV	-	-	-	0
Totals for Placebo Group	**38 Total: 23 BV, 10 Yeast, 5 Mixed**	Included in Prior Infections (pre-treatment)				**9 Total: 3 BV, 5 yeast, 1 mixed**

Summary of Infections Prior to and During Study - Zinc Group (N=15)

Partici-pant	Prior Infections	Initial Exam	3 month Exam	6 month Exam	Extra Visits	Totals Infections During Study
5756	2 Yeast	-	-	-	2 yeast	2 Yeast
7873	1 BV, 1 Yeast	BV	-	-	-	0
5846	3 BV	-	-	-	2 BV	2 BV
7854	1 BV, 1 Mixed	BV	BV	BV	-	2 BV
8990	2 BV	BV	BV	BV	-	2 BV
2316	4 BV, 4 Yeast	Mixed	BV	Mixed	2 Mixed	3 Mixed, 1 BV
5022	4 BV	BV	BV	-	-	1 BV
5916	3 Yeast	BV	-	BV	3 BV, 2 Mixed, 1 Yeast	4 BV, 2 Mixed, 1 Yeast

Partici-pant	Prior Infections	Initial Exam	3 month Exam	6 month Exam	Extra Visits	Totals Infections During Study
1419	2 BV	BV	BV	Mixed	-	1 BV, 1 Mixed
42	6 Yeast	Mixed	-	-	1 Yeast	1 Yeast
11131	2 BV	BV	-	-	-	0
5195	3 Yeast	-	BV	-	1 Yeast	1 Yeast, 1 BV
3238	2 BV	-	-	-	-	0
3256	1 BV, 1 Mixed, 1 K. pneumonia	-	-	-	-	0
10054	2 BV, 2 Mixed	-	-	-	-	0
Totals for Zinc Group	**57 total: 31 BV, 19 Yeast, 6 mixed, 1 other**	Included in Prior Infections (pre-treatment)				**26 Total: 14 BV, 6 Yeast, 6 Mixed**

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